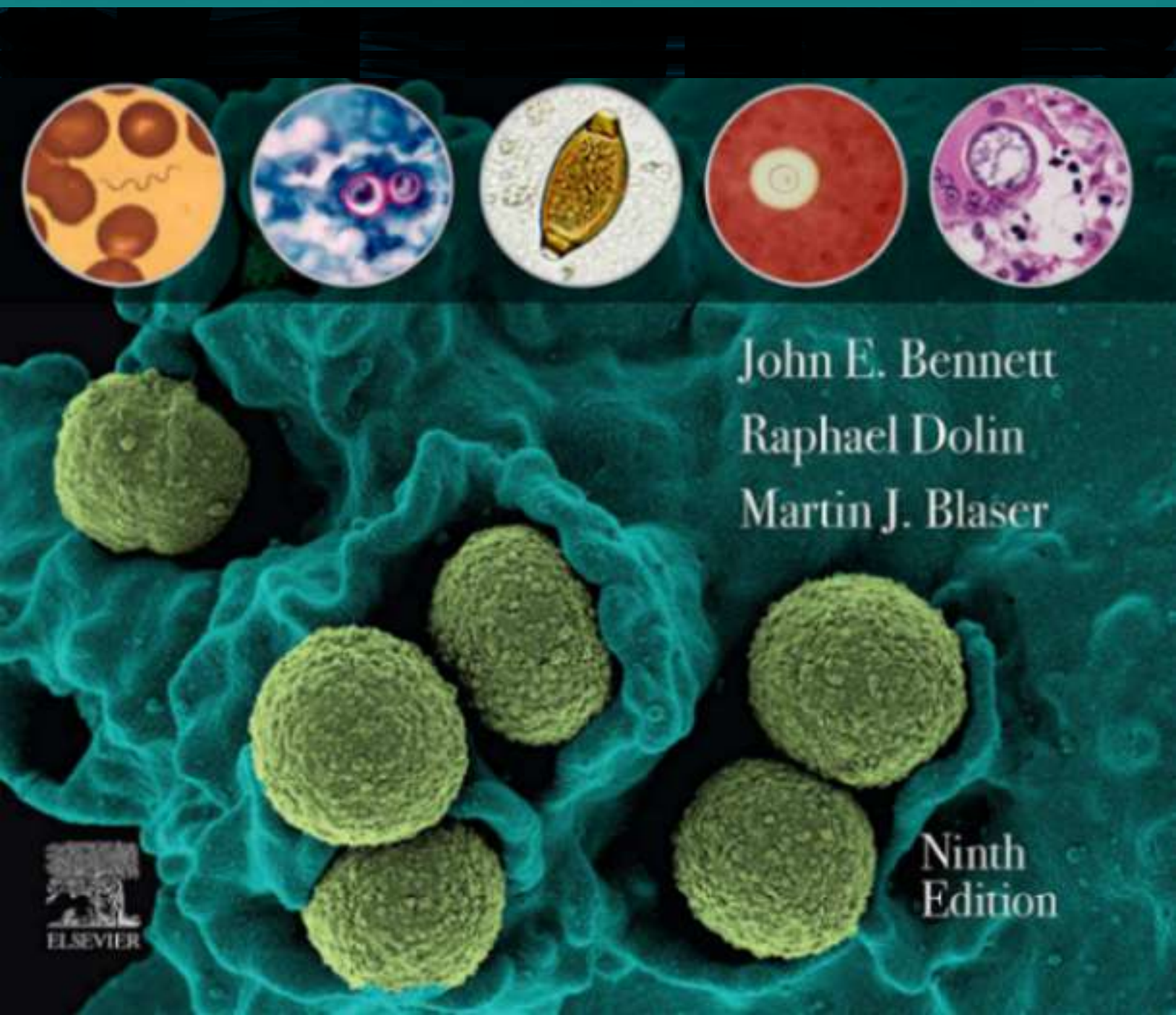
Mandell, Douglas, and Bennett's

Principles and Practice of Infectious Diseases



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Ninth Edition

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Principles and Practice of Infectious Diseases

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- Drugs for Protozoal Infections Other Than Malaria by James S. McCarthy, Glenn W. Wortmann, and Louis V. Kirchhoff
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- Introduction to Herpesviridae by Jeffrey I. Cohen
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- Infections Caused by Percutaneous Intravascular Devices by Susan E. Beekmann and David K. Henderson
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Ticks, Including Tick Paralysis

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Director, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland
Innate (General or Nonspecific) Host Defense Mechanisms

Jules L. Dienstag, MD
Carl W. Walter Professor of Medicine, Harvard Medical School; Physician, Massachusetts General Hospital, Boston, Massachusetts
Viral Hepatitis
Antiviral Drugs Against Hepatitis Viruses

Yohei Doi, MD, PhD
Associate Professor of Medicine, Division of Infectious Diseases, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
Ertapenem, Imipenem, Meropenem, Doripenem, and Aztreonam
Penicillins and β-Lactamase Inhibitors

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

Gerald R. Donowitz, MD
Professor of Medicine and Infectious Diseases/International Health, Department of Medicine, University of Virginia, Charlottesville, Virginia
Linezolid, Tedizolid, and Other Oxazolidinones

Curtis J. Donskey, MD
Professor of Medicine, Case Western Reserve School of Medicine; Staff Physician, Infectious Diseases Section, Cleveland VA Medical Center, Cleveland, Ohio
Clostridioides difficile (*Formerly* Clostridium difficile) *Infection*

Philip R. Dormitzer, MD, PhD
Vice President and Chief Scientific Officer Viral Vaccines, Pfizer, Pearl River, New York
Rotaviruses

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

Kathryn Dupnik, MD
Assistant Professor, Medicine, Weill Cornell Medicine, New York, New York
Leprosy (Mycobacterium leprae)

Herbert L. DuPont, MD
Professor of Infectious Diseases, University of Texas School of Public Health and Mary W. Kelsey Chair, University of Texas McGovern Medical School, Houston, Texas
Bacillary Dysentery: Shigella *and Enteroinvasive* Escherichia coli

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Prevention of Infective Endocarditis

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Associate Professor of Medicine, Harvard Medical School; Physician, Division of Infectious Diseases, Massachusetts General Hospital; Director, Infectious Disease Service, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts
Endophthalmitis
Introduction to Eye Infections
Periocular Infections
Infectious Causes of Uveitis

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Professor of Medicine, University of Paris-Diderot School of Medicine, Paris, France
Prevention of Infective Endocarditis

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Legionnaires' Disease and Pontiac Fever

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Professor of Medicine Emeritus, David Geffen School of Medicine at UCLA, Division of Infectious Diseases, Harbor-UCLA Medical Center, Senior Investigator, Los Angeles Biomedical Institute at Harbor UCLA, Los Angeles, California
Candida *Species*

Morven S. Edwards, MD
Professor of Pediatrics, Baylor College of Medicine; Attending Physician, Department of Pediatrics, Section of Infectious Diseases, Texas Children's Hospital, Houston, Texas
Streptococcus agalactiae *(Group B Streptococci)*

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Professor, Departments of Medicine, Microbiology, and Physiological Systems, Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, Massachusetts
Acute Pneumonia

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Innate (General or Nonspecific) Host Defense Mechanisms

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Chair, Department of Microbiology and Immunology, Professor of Medicine, State University of New York (SUNY) Upstate Medical University, Syracuse, New York
Flaviviruses (Dengue, Yellow Fever, Japanese Encephalitis, West Nile Encephalitis, Usutu Encephalitis, St. Louis Encephalitis, Tick-Borne Encephalitis, Kyasanur Forest Disease, Alkhurma Hemorrhagic Fever, Zika)

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Chronic Fatigue Syndrome (Systemic Exertion Intolerance Disease)

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Respiratory Syncytial Virus

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*Brucellosis (*Brucella *Species)*

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Mucosal Immunity

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Senior Safety Physician, Chief Medical Officer's Office, Oncology R&D, AstraZeneca, Gaithersburg, Maryland
Malaria (Plasmodium *Species*)

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Associate Professor of Medicine and Global Health, Emory University School of Medicine, Atlanta, Georgia
Tapeworms (Cestodes)

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

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Professor of Medicine, Department of Medicine, Division of Infectious Diseases, University of Rochester School of Medicine, Rochester, New York
Human Metapneumovirus

Anthony S. Fauci, MD
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The Immunology of Human Immunodeficiency Virus Infection

Thomas Fekete, MD
Professor of Medicine, Chair of Medicine, Temple University School of Medicine, Philadelphia, Pennsylvania
Bacillus *Species and Related Genera Other Than* Bacillus anthracis

Paul D. Fey, PhD
Professor, Department of Pathology and Microbiology, University of Nebraska Medical Center College of Medicine, Omaha, Nebraska
Staphylococcus epidermidis *and Other Coagulase-Negative Staphylococci*

†Deceased.

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Vesicular Stomatitis Virus and Related Vesiculoviruses (Chandipura Virus)

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Mycobacterium tuberculosis

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Pharyngitis

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Rickettsia akari *(Rickettsialpox)*

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Endocarditis and Intravascular Infections

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Infections in Returning Travelers
Protection of Travelers

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Bacillus anthracis *(Anthrax)*

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Coccidioidomycosis (Coccidioides *Species*)

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Evaluation of the Patient With Suspected Immunodeficiency

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Human Immunodeficiency Viruses

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Human Immunodeficiency Virus Infection in Women

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

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Blastomycosis

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Chlamydia pneumoniae

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Brain Abscess

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Marburg and Ebola Virus Hemorrhagic Fevers

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Babesia *Species*

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Topical Antibacterials

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

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Rubella Virus (German Measles)
Measles Virus (Rubeola)

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Robert P. Kelch Research Professor Emerita of Pediatrics, University of Michigan Medical School and C.S. Mott Children's Hospital, Ann Arbor, Michigan
Infections in Asplenic Patients

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Microbial Keratitis

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Outpatient Parenteral Antimicrobial Therapy

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Bites

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Paracoccidioidomycosis

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Esophagitis

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Infectious Diseases and Microbiology Departments, Austin Health, Department of Epidemiology and Preventive Medicine, Monash University; Department of Medicine, University of Melbourne, Melbourne, Australia
Fusidic Acid

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Stenotrophomonas maltophilia *and* Burkholderia cepacia *Complex*

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Enterobacteriaceae

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Foodborne Disease

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Antimycobacterial Agents

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Thomas H. Hunter Professor of International Medicine, Founding Director, Center for Global Health, Division of Infectious Diseases and International Health, University of Virginia School of Medicine, Charlottesville, Virginia
Diarrhea With Little or No Fever
Acute Dysentery Syndromes (Diarrhea With Fever)

Hanefi C. Gul, MD
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*Brucellosis (*Brucella *Species)*

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Leptospira *Species (Leptospirosis)*

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Mycobacterium tuberculosis

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Infections in Solid-Organ Transplant Recipients

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Microbial Keratitis

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Bordetella pertussis

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Chlamydia pneumoniae

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Entamoeba *Species, Including Amebic Colitis and Liver Abscess*

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

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Coxiella burnetii *(Q Fever)*

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Approach to the Patient With Central Nervous System Infection
Acute Meningitis

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Associate Professor, Department of Infectious Diseases, Northwestern University Feinberg School of Medicine, Chicago, Illinois
Hepatitis B Virus
Hepatitis Delta Virus

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Dermatophytosis (Ringworm) and Other Superficial Mycoses

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Infections Caused by Percutaneous Intravascular Devices

Kevin P. High, MD, MS
Professor of Medicine and Translational Science, Internal Medicine, Wake Forest School of Medicine; President, Wake Forest Baptist Health, Winston-Salem, North Carolina
Infections in Older Adults

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Human Genetics and Infection

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The Task Force for Global Health, Center for Vaccine Equity, Decatur, Georgia
Immunization

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Antiretroviral Therapy for Human Immunodeficiency Virus Infection

Sarah Hochman, MD
Associate Hospital Epidemiologist, Infection Prevention and Control, NYU Langone Health; Assistant Professor, Department of Medicine, Division of Infectious Diseases and Immunology, NYU School of Medicine, New York, New York
Acinetobacter *Species*

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Professor of Medicine, University of Lorraine School of Medicine, Nancy, France
Prevention of Infective Endocarditis

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Chief, Infectious Disease Service, Associate Member, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York
Cell-Mediated Defense Against Infection

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Evaluation of the Patient With Suspected Immunodeficiency

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Endocarditis and Intravascular Infections

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Mycoplasma pneumoniae *and Atypical Pneumonia*

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Quinolones

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Professor of Clinical Medicine, Department of Medicine, Clinical Director, Division of Infectious Diseases, University of Miami Miller School of Medicine; Chief of Medicine, Miami VA Health System, Miami, Florida
Health Care–Associated Urinary Tract Infections

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Chronic Meningitis

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Professor of Clinical Medicine, Weill Cornell School of Medicine, New York, New York; Chief of Service, Infectious Diseases, New-York Presbyterian Brooklyn Methodist Hospital, Brooklyn, New York
Acute Exacerbations of Chronic Obstructive Pulmonary Disease

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Division of Infectious Diseases, Department of Internal Medicine, Carolinas Medical Center, Charlotte, North Carolina
Urinary Tract Agents: Nitrofurantoin, Fosfomycin, and Methenamine
Relapsing Fever Caused by Borrelia *Species*

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Adjunct Professor of Medicine, Department of Medicine, Infectious Disease Division, University of Texas Health Science Center at San Antonio; Partner, San Antonio Infectious Diseases Consultants, San Antonio, Texas
Agents of Chromoblastomycosis
Agents of Mycetoma
Uncommon Fungi and Related Species

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Dean, National School of Tropical Medicine; Professor, Pediatrics and Molecular & Virology and Microbiology; Head, Section of Pediatric Tropical Medicine, Baylor College of Medicine, Texas Children's Hospital Endowed Chair of Tropical Pediatrics; Director, Sabin Vaccine Institute, Texas Children's Hospital Center for Vaccine Development; University Professor, Department of Biology, Baylor University; President, Sabin Vaccine Institute, Baker Institute, Fellow in Disease and Poverty, Rice University; Co-Editor-in-Chief, PLoS Neglected Tropical Diseases, Houston, Texas
Intestinal Nematodes (Roundworms)

Noreen A. Hynes, MD, MPH, DTM&H
Associate Professor of Medicine (Infectious Diseases), School of Medicine and International Health (Global Epidemiology and Control), Bloomberg School of Public Health, Johns Hopkins University; Associate Medical Director, Biocontainment Unit (BCU), Johns Hopkins Hospital, Baltimore, Maryland
Bioterrorism: An Overview

Nicole M. Iovine, MD, PhD
Associate Professor of Medicine, University of Florida; Hospital Epidemiologist, UF Health, Gainesville, Florida
Campylobacter jejuni *and Related Species*

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Professor of Medicine and Surgery, Northwestern University Feinberg School of Medicine, Chicago, Illinois
Parainfluenza Viruses

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Department of Pediatrics, Division of Infectious Diseases, Emory University; Children's Healthcare of Atlanta, Atlanta, Georgia
Nonsuppurative Poststreptococcal Sequelae: Rheumatic Fever and Glomerulonephritis

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Capnocytophaga

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Streptococcus pneumoniae

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Emerging and Reemerging Infectious Disease Threats

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

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Listeria monocytogenes

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The Acutely Ill Patient With Fever and Rash

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Health Care–Acquired Hepatitis

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William Ellery Channing Professor of Medicine and Professor of Microbiology and Immunobiology, Division of Immunology, Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts
Anaerobic Infections: General Concepts

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Polymyxins (Polymyxin B and Colistin)

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Polymyxins (Polymyxin B and Colistin)

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

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

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

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Emerging and Reemerging Infectious Disease Threats

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Antibiotic Allergy

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Hepatitis A Virus

Rose Kim, MD
Assistant Dean for Faculty Affairs, Associate Professor of Medicine, Department of Medicine, Cooper Medical School of Rowan University, Camden, New Jersey
Other Coryneform Bacteria, Arcanobacterium haemolyticum, *and Rhodococci*

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Tapeworms (Cestodes)

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

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Campylobacter jejuni *and Related Species*

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Mucosal Immunity

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Blastomycosis

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Nosocomial Pneumonia

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Prosthetic Valve Endocarditis

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Myocarditis and Pericarditis

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Bartonella, *Including Cat-Scratch Disease*

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Chlamydia pneumoniae

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Anaerobic Cocci and Anaerobic Gram-Positive Nonsporulating Bacilli

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Frances King Black Endowed Professor, Department of Infectious Diseases, Division of Internal Medicine; Deputy Head, Division of Internal Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas
Agents of Mucormycosis and Entomophthoramycosis

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

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Introduction to Protozoal Diseases

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Free-Living Amebae

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Toxoplasma gondii

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Immunization

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Transfusion- and Transplantation-Transmitted Infections

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Microbial Conjunctivitis

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Antibiotic Allergy

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Syndromes of Enteric Infection

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Outpatient Parenteral Antimicrobial Therapy

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Mycoplasma pneumoniae *and Atypical Pneumonia*

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Aminoglycosides

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Cephalosporins

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

†Deceased.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Aurora Pop-Vicas, MD, MPH
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Molecular Mechanisms of Antibiotic Resistance in Bacteria

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Kathryn L. Ruoff, PhD
Research Scientist, O'Toole Lab, Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire
Classification of Streptococci

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Mediastinitis
Staphylococcus epidermidis *and Other Coagulase-Negative Staphylococci*

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Rabies (Rhabdoviruses)

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Agents of Actinomycosis

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Disinfection, Sterilization, and Control of Hospital Waste

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Typhoid Fever, Paratyphoid Fever, and Typhoidal Fevers
Vibrio cholerae

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Temperature Regulation and the Pathogenesis of Fever

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Syphilis (Treponema pallidum)

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Corynebacterium diphtheriae *(Diphtheria)*

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Hyperbaric Oxygen

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Myocarditis and Pericarditis

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Pulmonary Manifestations of Human Immunodeficiency Virus Infection

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Herpes Simplex Virus

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Psittacosis (*Due to* Chlamydia psittaci)

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Whipple Disease

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Trichomonas vaginalis

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Prebiotics, Probiotics, and Synbiotics

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Acute Exacerbations of Chronic Obstructive Pulmonary Disease

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

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Nonsuppurative Poststreptococcal Sequelae: Rheumatic Fever and Glomerulonephritis

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

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

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Mycoplasma pneumoniae *and Atypical Pneumonia*

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Diagnosis of Human Immunodeficiency Virus Infection

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Infections in Solid-Organ Transplant Recipients

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Free-Living Amebae

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Pneumocystis *Species*

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Urinary Tract Infections

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Infections of Nonvalvular Cardiovascular Devices

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Nocardia *Species*

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Osteomyelitis

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Lyme Disease (Lyme Borreliosis) Due to Borrelia burgdorferi

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Other Gram-Negative and Gram-Variable Bacilli

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Neisseria meningitidis

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Adenoviruses

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Mycobacterium tuberculosis

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Antifungal Agents: Amphotericin B

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Streptococcus pyogenes

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Klebsiella granulomatis *(Donovanosis, Granuloma Inguinale)*

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Quinolones

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Streptococcus anginosus *Group*

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Mycobacterium avium *Complex*

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

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

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Surgical Site Infections and Antimicrobial Prophylaxis

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

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Hepatitis Delta Virus

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Antifungal Drugs: Azole

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

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

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Fusidic Acid

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

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

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Infections in Recipients of Hematopoietic Stem Cell Transplants

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Infections in the Immunocompromised Host: General Principles

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Mediastinitis

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Babesia *Species*

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Hepatitis A Virus

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The Human Microbiome of Local Body Sites and Their Unique Biology

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Parechoviruses

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Prophylaxis and Empirical Therapy of Infection in Cancer Patients

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Sinusitis

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Vibrio cholerae

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

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

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Acute Bronchitis
Respiratory Syncytial Virus

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Pneumocystis *Species*

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Tropical Sprue and Environmental Enteric Dysfunction

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Nocardia *Species*

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Intestinal Nematodes (Roundworms)

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

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Epiglottitis
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Cryptosporidiosis (Cryptosporidium *Species*)

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Infections of Nonvalvular Cardiovascular Devices

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Endemic Treponematoses

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Mycobacterium avium *Complex*

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Foodborne Disease

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Drugs for Protozoal Infections Other Than Malaria

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Fever of Unknown Origin

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Pasteurella *Species*

Preface to the 9th Edition

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

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

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

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

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

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

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Basic Principles in the Diagnosis and Management of Infectious Diseases

I

A Microbial Pathogenesis

1

A Molecular Perspective of Microbial Pathogenicity

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

ATTRIBUTES OF MICROBIAL PATHOGENS

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

TABLE 1.2 Attributes Shared by Bacterial Pathogens

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

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

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

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

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

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

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

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

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

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

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

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

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

EVOLUTION OF BACTERIAL PATHOGENICITY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

REGULATION OF BACTERIAL PATHOGENICITY

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

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

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

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

TABLE 1.4 Examples of Bacterial Virulence Regulatory Systems

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

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

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

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

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

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

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

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

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

CLOSE ENCOUNTERS: PATHOGENS AS CELL BIOLOGISTS

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

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

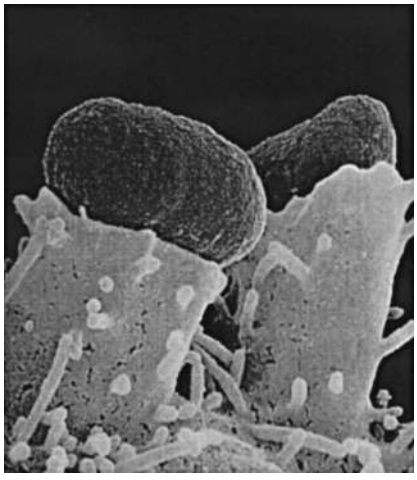

FIG. 1.1 Scanning electron micrograph depicting pseudopod, or "pedestal," formation by enteropathogenic *escherichia coli* (EPEC) as it interacts with the surface of an epithelial cell. This form of intimate adherence requires a bacterial adhesin, intimin; a receptor of bacterial origin, Tir, that is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

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

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

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

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

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

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

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

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

IDENTIFICATION AND CHARACTERIZATION OF VIRULENCE GENES

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

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

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

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

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

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

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

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

DEDICATION

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

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The complete reference list is available online at Expert Consult.

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

Kjersti Aagaard, Ruth Ann Luna, and James Versalovic

DEFINING THE HUMAN MICROBIOME

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

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

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

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

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

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

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

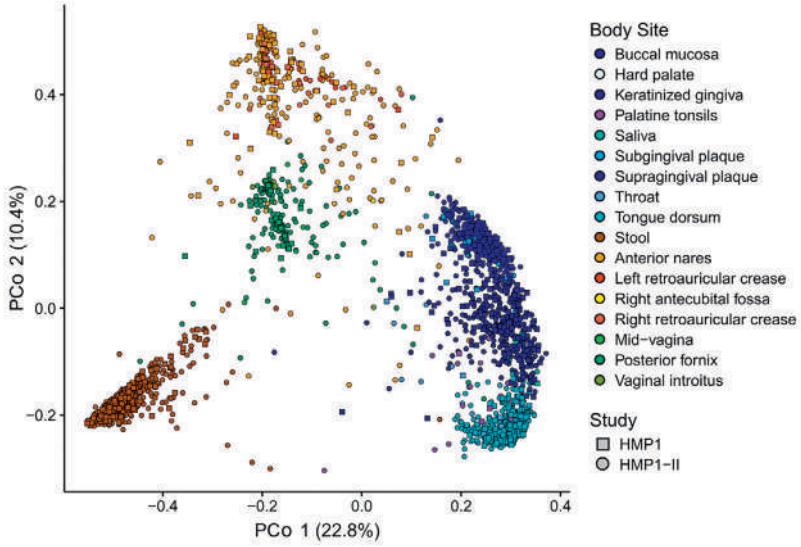

FIG. 2.1 The human microbiome is composed of distinct bacterial populations at different body sites. This principal components *(PCo)* analysis plot shows each distinct body site (indicated by distinct colors) and its microbial composition in healthy adults. Each colored circle in space represents an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

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

From Whence and When Do Our Microbiomes Come?

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

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

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

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

TABLE 2.1 Explanation of Key Terms

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

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

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

ORAL MICROBIOME

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

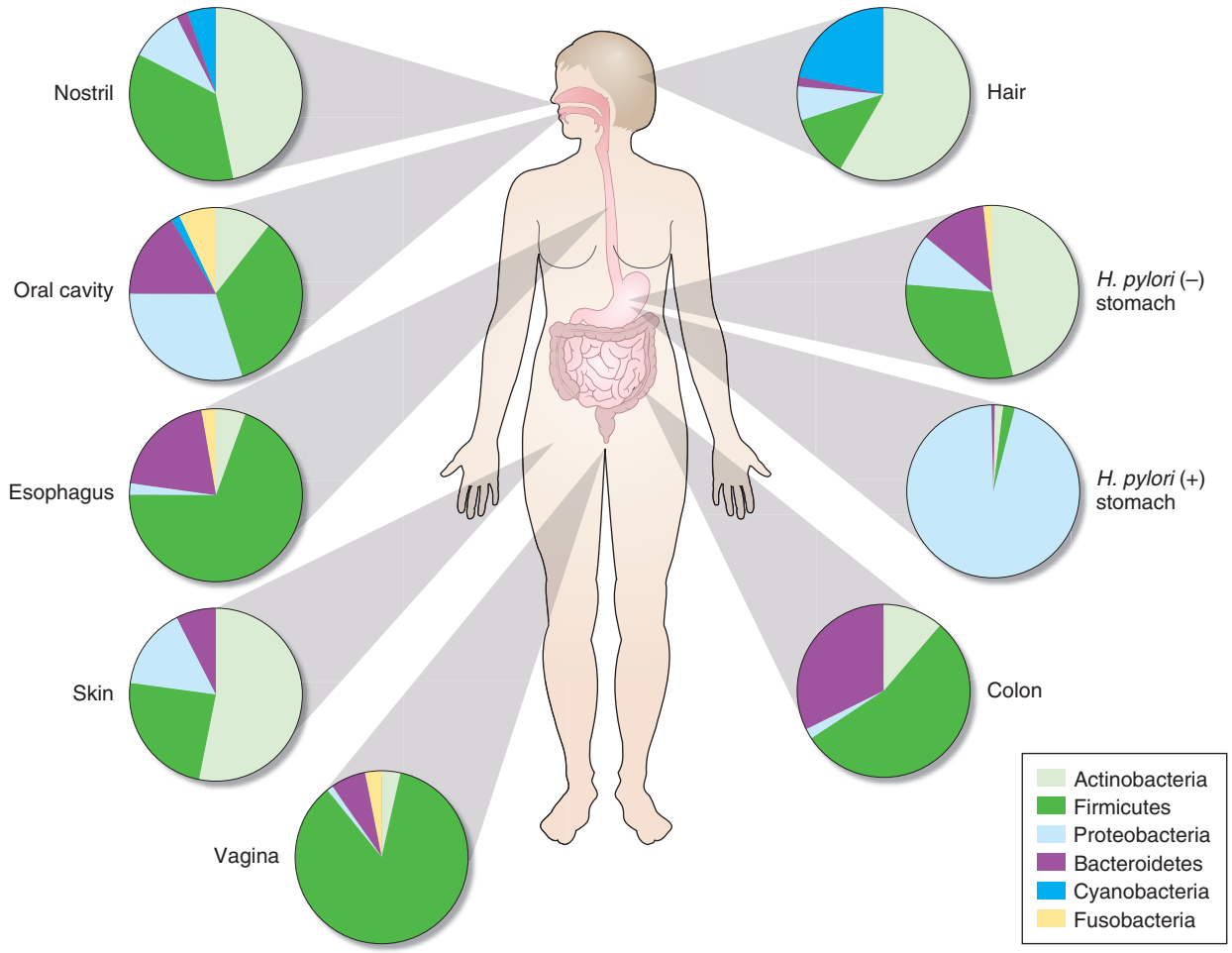

FIG. 2.2 Compositional differences in the microbiome by anatomic site. Metagenomic massively parallel sequencing approaches have demonstrated exquisite body site specificity, and higher level (e.g., phylum) taxonomic features display temporal (longitudinal) stability in individuals at specific anatomic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

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

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

Associations Between Oral Microbiota and Disease States

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

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

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

SKIN AND NASOPHARYNX

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

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

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

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

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

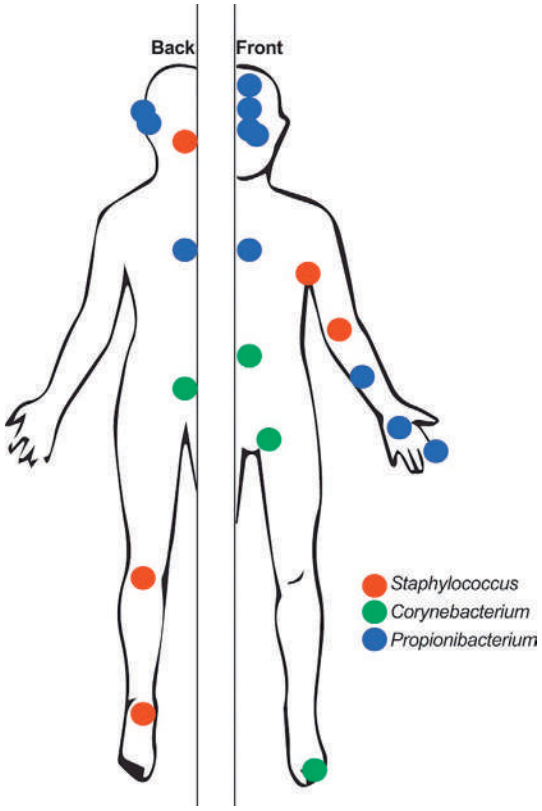

FIG. 2.3 Predominant microbes in specific skin sites. This schematic figure shows the predominant bacterial genera (by color) at each skin site on the human body. *(From Grice EA. The intersection of microbiome and host at the skin interface: genomic- and metagenomic-based insights.* Genome Res. *2015;25:1514–1520.)*

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

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

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

AIRWAY AND PULMONARY MICROBIOME

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

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

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

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

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

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

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

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

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

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

GASTROINTESTINAL TRACT

Esophagus

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

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

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

Stomach

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

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

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

Intestine (Small and Large)

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

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

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

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

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

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

VAGINAL MICROBIOME

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

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

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

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

GROUP B STREPTOCOCCUS

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

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

THE HUMAN MICROBIOME DURING PREGNANCY

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

SUMMARY AND FUTURE DIRECTIONS

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

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

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3 Prebiotics, Probiotics, and Synbiotics

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SHORT VIEW SUMMARY

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

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

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

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

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

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

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

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

ISSUES REGARDING THE COMPLEXITY AND VARIABILITY OF PROBIOTICS

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

TABLE 3.1 Concerns About Marketed Probiotic Formulations

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

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

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

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

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

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

CLINICAL STUDIES OF PROBIOTICS

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

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

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

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

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

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

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

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

PROPOSED MECHANISMS OF ACTION OF PROBIOTICS

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

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

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

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

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

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

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

POTENTIAL ADVERSE EFFECTS OF PROBIOTIC THERAPY

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

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

TABLE 3.4 Populations Potentially at Risk of Harm From Probiotic Ingestion

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

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

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

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

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

SUMMARY AND FUTURE DIRECTIONS

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

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B Host Defense Mechanisms

4 Innate (General or Nonspecific) Host Defense Mechanisms

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

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

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

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

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

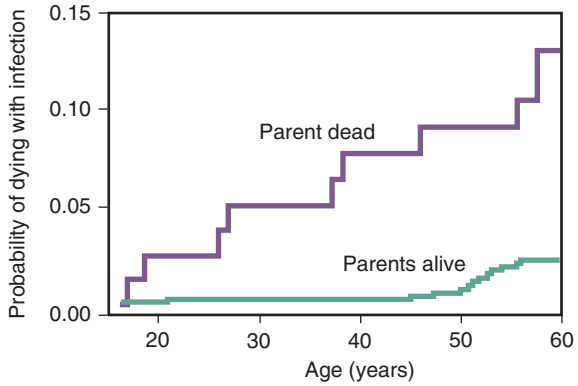

FIG. 4.1 Probability of dying from an infection before a given age for adoptees with at least one biologic parent who died before age 50 years (parent dead) of an infection versus adoptees whose biologic parents were alive at that age (parents alive). *(From Sorensen TIA, Neilson GG, Anderson PK, et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

TABLE 4.1 Features of Innate and Adaptive Immunity

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

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

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

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

Skin

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

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

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

Mucous Membranes

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

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

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

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

Respiratory Tract

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

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

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

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

Gastrointestinal Tract

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

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

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

Genitourinary Tract

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

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

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

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

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

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

Eye

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

INNATE IMMUNE SENSING OF INVADING PATHOGENS

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

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

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

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

Phagocytosis

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

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

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

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

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

Autophagy

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

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

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

The Inflammatory Response

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

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

Cytokines and Chemotaxis

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

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

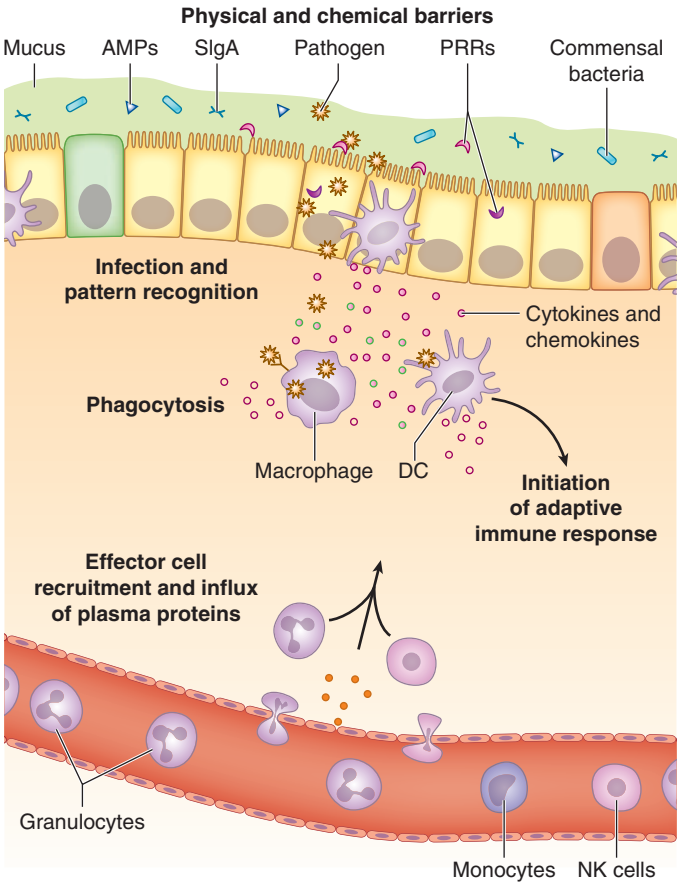

FIG. 4.2 Overview of innate immune defenses. Mucosal surfaces comprise epithelial cells interspersed with mucus-secreting cells and other cell types that produce antimicrobial factors and sample luminal antigens. Mucus carries antimicrobial peptides and proteins *(AMPs),* immunoglobulins such as secretory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

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

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

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

PATHOGEN INTERFERENCE WITH INNATE IMMUNE RESPONSES

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

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

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

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

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5 Adaptive Immunity: Antibodies and Immunodeficiencies

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SHORT VIEW SUMMARY

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

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

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

IMMUNOGLOBULIN STRUCTURE

Basic Antibody Structure

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

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

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

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

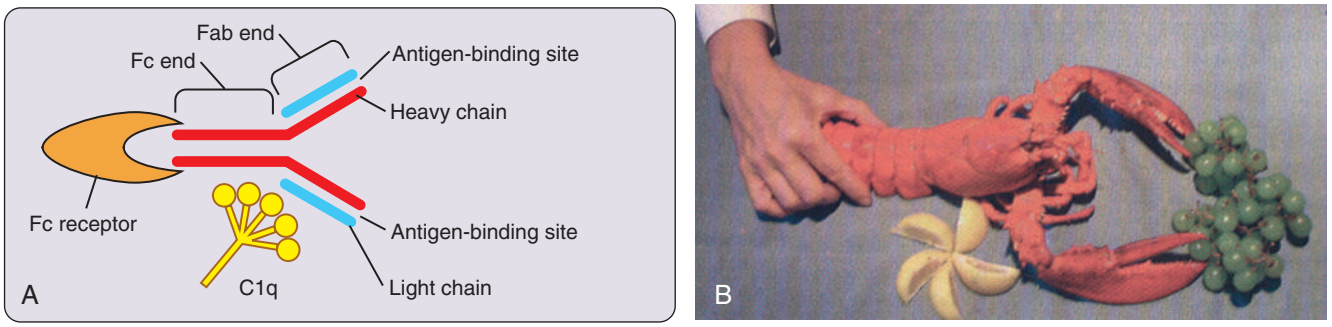

FIG. 5.1 Structure of antibodies. (A) Antibody molecules are composed of two heavy chains *(red lines)* and two light chains *(blue lines)* held together by disulfide bonds. The two heavy chains join to form a tail (Fc end), which can interact with Fc receptors on a variety of cells. The heavy and light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

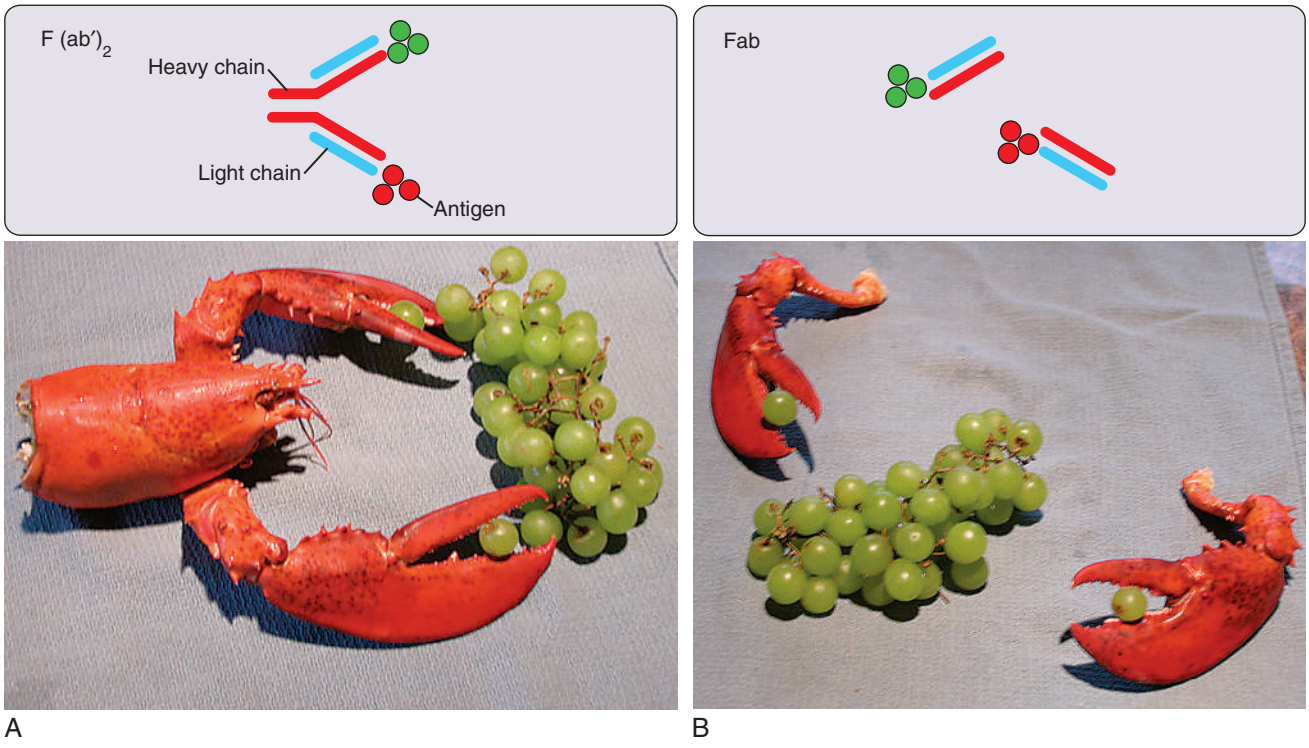

FIG. 5.2 Cleavage fragments of antibodies. (A) Papain digests the immunoglobulin molecule into an F(ab′)$_2$ fragment. This fragment is still dimeric, but it can no longer interact with Fc receptors on cells. Under reducing conditions, the disulfide bonds holding the two heavy chains together can be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

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

F(ab′)$_2$, Fab, and Fc Pieces

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

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

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

Antigen Binding, Affinity, and Avidity

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

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

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

Immunoglobulin Classes

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

Immunoglobulin M

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

Immunoglobulin G

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

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

TABLE 5.1 Characteristics of the Immunoglobulin Classes

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

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

Immunoglobulin A

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

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

Immunoglobulin D

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

Immunoglobulin E

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

EFFECTOR FUNCTIONS MEDIATED BY ANTIBODIES

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

Blocking or Neutralization

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

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

Complement Activation

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

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

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

Opsonization

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

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

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

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

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