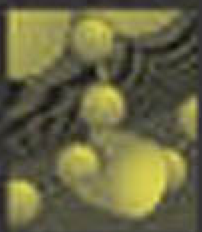
Kenneth J. Ryan / C. George Ray

Champoux • Drew • Neidhardt • Plorde

# *Sherris* MEDICAL MICROBIOLOGY

## An Introduction to Infectious Diseases




Fourth Edition

4TH EDITION

# SHERRIS MEDICAL MICROBIOLOGY

AN INTRODUCTION TO INFECTIOUS DISEASES

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## AN INTRODUCTION TO INFECTIOUS DISEASES

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*Dedication*

*To Fritz*[a]

[a] Fritz D. Schoenknecht, MD, American microbiologist (1931–1996). Your wit, intellect, music, and twinkle-eyed warnings remain a cherished part of our lives.

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# CONTENTS

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# PREFACE

With this fourth edition, *Sherris Medical Microbiology,* which began almost two decades ago as *Medical Microbiology* (1984), retains the same team as the third edition with some redistribution in assignments. The most significant of these is the decision of George Ray to join Ken Ryan as editor. John Sherris continues to act as an advisor to all of us.

The goal of *Sherris Medical Microbiology* remains unchanged from that of the first edition. This book is intended to be the primary text for students of medicine and medical science who are encountering microbiology and infectious diseases for the first time. The organization is the same as the third edition with basic topics followed by chapters on the major bacterial, viral, fungal, and parasitic pathogens. We have tried to strengthen the pathogen presentation style introduced in the third edition. For each virus, bacterium, fungus, or parasite, the most important features of the organism (structure, metabolism, genetics), the disease (epidemiology, pathogenesis, immunity), and the clinical aspects (manifestations, diagnosis, treatment, prevention) are placed in distinct sections and in the same order. The opening to each of these sections is now marked by an icon for the organism , disease , or clinical aspects . At the juncture between the organism and disease sections, a new feature, the Clinical Capsule, has been introduced. This brief snapshot of the disease is intended to orient the first-time reader before they dive into discussions of pathogenic mechanisms. Fourteen brief chapters at the end summarize the relevant clinical, diagnostic, and therapeutic information into the most common clinical infectious syndromes without the addition of new material. It is hoped that these chapters will be of particular value when the student prepares for case discussions or sees patients.

In *Sherris Medical Microbiology,* the emphasis is on the text narrative, which is designed to be read comprehensively, not as a reference work. In this regard all the pathogenic microorganisms we feel are important are included at a level of detail relevant for medical students. Any added detail in tables and figures is for example or explanation and not intended to be learned. Marginal notations throughout the text have been revised to capsulize major points as an aid for the student during review. A student scanning the red marginal notes will encounter all the major points in a chapter. If a note looks unfamiliar, the relevant text is immediately adjacent.

An overview chapter on the immune response to infection is included for continuity, but it is assumed this subject will be covered by one of the many excellent immunology texts available. The chapter on dental microbiology has been updated to serve the needs of dental students.

Much new material has been included, but in order to keep the student from being overwhelmed, older or less important information has been deleted to keep the size of this book approximately the same as the previous edition. As a rule of thumb, material on classic microbial structures, toxins, and the like has been trimmed unless its role in disease will be explained in the following sections. At the same time, we have tried not to eliminate detail to the point of becoming synoptic and uninteresting. For example, adequate explanation of the pathogenesis of an infectious disease may require discussion of the roles played by multiple proteins, genes, and regulators. Where these features form a coherent picture we have tried to tell the complete story, particularly if it is instructive as a general principle. When details such as the names of proteins and genes have been placed in parentheses, it is a sign the authors feel they need not be memorized.

A saving grace is that our topic is important, dynamic, and fascinating. Who could have predicted that AIDS, which occupied less than a page in the first edition, would in the 1990s become the leading cause of death in young American men and, with this edition, enter a period of drug suppression and hope? Gastritis and ulcers attributed to stress in the past are now being cured by antimicrobial therapy directed against *Helicobacter pylori,* but this bacterium has now been officially declared a carcinogen due to additional links with gastric cancer. Just as we were about to hit the presses, an apparently new infectious disease emerged from the Far East in the form of the severe acute respiratory syndrome (SARS). Never a dull moment! These and many other infectious agents and diseases old and new are described and explained in these pages. The student is invited to read them and begin a lifetime of learning in microbiology, infectious diseases, and medicine.

***Kenneth J. Ryan***
***C. George Ray***
Editors

# ACKNOWLEDGMENTS

The authors wish to thank Drs. Steve Moseley and Irene Weitzman for selected chapter review and helpful suggestions. Administrative support and manuscript review were provided by Diane Ray, Hildi Williams, Carol Wertman, and Alexa Suslow. We also wish to acknowledge the professionalism of Janet Foltin, Karen Davis, and the McGraw-Hill staff, who took on this complicated new project and completed it with remarkable speed and flexibility.

New illustrations for this edition were prepared under the direction of Becky Hainz-Baxter and Alexander Teshin Associates, whose skill and ability to respond creatively to the diverse needs of this text are gratefully acknowledged. Illustrations prepared by Sam Eng for the mycology and parasitology sections in the first edition have been carried over to this edition, as have many of the illustrations prepared for the second edition by Marilyn Pollack-Senura, and for the third edition by Cindy Tinnes.

Finally, we wish to acknowledge our students, past and present, who provide the stimulation for continuation of this work, and our families who provide the encouragement and support that make it possible.

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4th Edition

# Sherris Medical Microbiology

An Introduction to Infectious Diseases

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CHAPTER 1

# Overview

KENNETH J. RYAN

> Humanity has but three great enemies: fever, famine and war; of these by far the greatest, by far the most terrible, is fever.
>
> SIR WILLIAM OSLER, 1896*

When Sir William Osler, the great physician/humanist wrote these words, fever (infection) was indeed the scourge of the world. Tuberculosis and other forms of pulmonary infection were the leading causes of premature death among the well to do and the less fortunate. The terror was due to the fact that although some of the causes of infection were being discovered, little could be done to prevent or alter the course of disease. In the 20th century, advances in public sanitation and the development of vaccines and antimicrobials changed this fact (Fig 1-1), but only for the nations that could afford the improvements. As the 21st century begins, the world is divided into countries in which heart attacks, cancer, and stroke have surpassed infection as a cause of death and those in which infection is still the leading cause of death.

A new uneasiness that is part evolutionary, part discovery, and part diabolic has taken hold. Infectious agents once conquered have demonstrated resistance to established therapy, such as multiresistant *Mycobacterium tuberculosis,* and new diseases, such as acquired immunodeficiency syndrome (AIDS), have emerged. The spectrum of infection has widened, with discoveries that organisms once thought to be harmless can cause disease under certain circumstances. Who could have guessed that *Helicobacter pylori,* not even mentioned in the first edition of this book, would be the major cause of gastric and duodenal ulcers and an officially declared carcinogen? Finally, bioterrorist forces have unearthed two previously controlled infectious diseases, anthrax and smallpox, and threatened their distribution as agents of biological warfare. For students of medicine, understanding the fundamental basis of infectious diseases has more relevance than ever.

## BACKGROUND

The science of medical microbiology dates back to the pioneering studies of Pasteur and Koch, who isolated specific agents and proved that they could cause disease by introducing the experimental method. The methods they developed lead to the first golden age of microbiology (1875–1910), when many bacterial diseases and the organisms responsible for them were defined. These efforts, combined with work begun by Semmelweis and Lister, which showed how these diseases spread, led to the great advances in public health that initiated the decline in disease and death. In the first half of the 20th century, scientists studied the structure, physiology, and genetics of microbes in detail and began to

*Osler W. *JAMA* 1896;26:999.

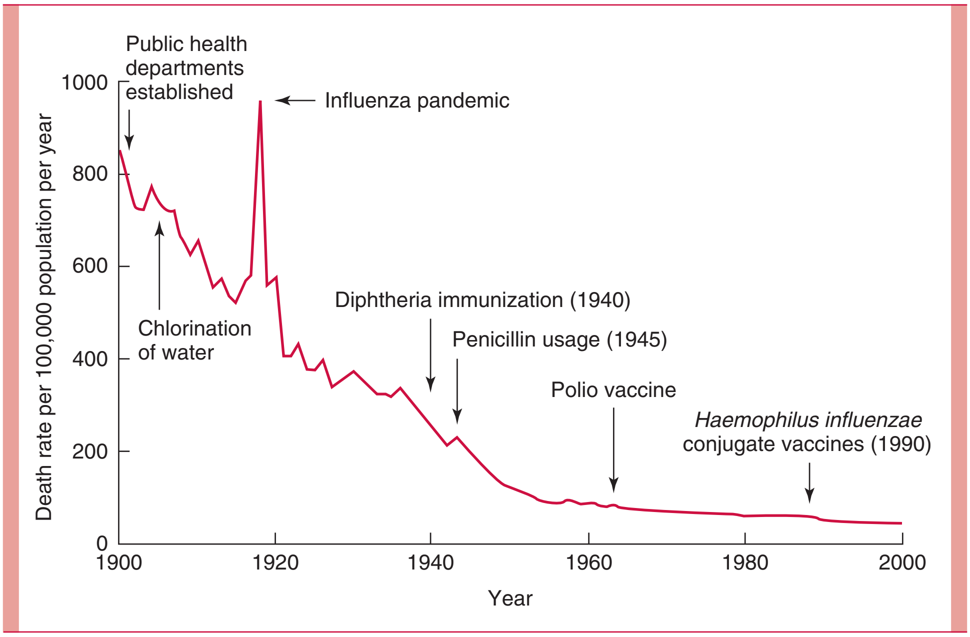


FIGURE 1–1
Death rates for infectious disease in the United States in the 20th century. Note the steady decline in death rates related to the introduction of public health, immunization, and antimicrobial interventions.

answer questions relating to the links between specific microbial properties and disease. By the end of the 20th century, the sciences of molecular biology, genetics, genomics, and proteomics extended these insights to the molecular level. Genetic advances have reached the point where it is possible to know not only the genes involved but understand how they are regulated. The discoveries of penicillin by Fleming in 1929 and of sulfonamides by Domagk in 1935 opened the way to great developments in chemotherapy. These gradually extended from bacterial diseases to fungal, parasitic, and finally viral infections. Almost as quickly, virtually all categories of infectious agents developed resistance to all categories of antimicrobics to counter these chemotherapeutic agents.

## THE INFECTIOUS AGENTS: THE MICROBIAL WORLD

Microbes are small

Microbiology is a science defined by smallness. Its creation was made possible by the invention of the microscope (Gr. *micro,* small + *skop,* to look, see), which allowed visualization of structures too small to see with the naked eye. This definition of microbiology as the study of microscopic living forms still holds if one can accept that some organisms can live only in other cells (eg, all viruses, some bacteria) and others have macroscopic forms (eg, fungal molds, parasitic worms).

Most play benign roles in the environment

Microorganisms are responsible for much of the breakdown and natural recycling of organic material in the environment. Some synthesize nitrogen-containing compounds that contribute to the nutrition of living things that lack this ability; others (oceanic algae) contribute to the atmosphere by producing oxygen through photosynthesis. Because microorganisms have an astounding range of metabolic and energy-yielding abilities, some can exist under conditions that are lethal to other life forms. For example, some bacteria can oxidize inorganic compounds such as sulfur and ammonium ions to generate energy, and some can survive and multiply in hot springs at temperatures above 75°C.

Products of microbes contribute to the atmosphere

Some microbial species have adapted to a symbiotic relationship with higher forms of life. For example, bacteria that can fix atmospheric nitrogen colonize root systems of legumes and of a few trees such as alders and provide the plants with their nitrogen requirements. When these plants die or are plowed under, the fertility of the soil is enhanced by nitrogenous compounds originally derived from the metabolism of the bacteria.

TABLE 1–1

**Distinctive Features of Prokaryotic and Eukaryotic Cells**

| Cell Component | Prokaryotes | Eukaryotes |
|---|---|---|
| Nucleus | No membrane, single circular chromosome | Membrane bounded, a number of individual chromosomes |
| Extrachromosomal DNA | Often present in form of plasmid(s) | In organelles |
| Organelles in cytoplasm | None | Mitochondria (and chloroplasts in photosynthetic organisms) |
| Cytoplasmic membrane | Contains enzymes of respiration; active secretion of enzymes; site of phospholipid and DNA synthesis | Semipermeable layer not possessing functions of prokaryotic membrane |
| Cell wall | Rigid layer of peptidoglycan (absent in *Mycoplasma*) | No peptidoglycan (in some cases cellulose present) |
| Sterols | Absent (except in *Mycoplasma*) | Usually present |
| Ribosomes | 70 S in cytoplasm | 80 S in cytoplasmic reticulum |

Ruminants can use grasses as their prime source of nutrition, because the abundant flora of anaerobic bacteria in the rumen break down cellulose and other plant compounds to usable carbohydrates and amino acids and synthesize essential nutrients including some amino acids and vitamins. These few examples illustrate the protean nature of microbial life and their essential place in our ecosystem.

The major classes of microorganisms in terms of ascending size and complexity are viruses, bacteria, fungi, and parasites. Parasites exist as single or multicellular structures with the same eukaryotic cell plan of our own cells. Fungi are also eukaryotic but have a rigid external wall that makes them seem more like plants than animals. Bacteria also have a cell wall, but their cell plan is prokaryotic (Table 1–1) and lacks the organelles of eukaryotic cells. Viruses have a genome and some structural elements but must take over the machinery of another living cell (eukaryotic or prokaryotic) in order to replicate.

Increasing complexity: viruses → bacteria → fungi → parasites

## Viruses

Viruses are strict intracellular parasites of other living cells, not only of mammalian and plant cells, but also of simple unicellular organisms, including bacteria (the bacteriophages). Viruses are simple forms of replicating, biologically active particles that carry genetic information in either DNA or RNA molecules, but never both. Most mature viruses have a protein coat over their nucleic acid and sometimes a lipid surface membrane derived from the cell they infect. Because viruses lack the protein-synthesizing enzymes and structural apparatus necessary for their own replication, they bear essentially no resemblance to a true eukaryotic or prokaryotic cell.

Viruses contain little more than DNA or RNA

Viruses replicate by using their own genes to direct the metabolic activities of the cell they infect to bring about the synthesis and reassembly of their component parts. A cell infected with a single viral particle may thus yield many thousands of viral particles, which can be assembled almost simultaneously under the direction of the viral nucleic acid. With many viruses, cell death and infection of other cells by the newly formed viruses result. Sometimes, viral reproduction and cell reproduction proceed

Replication is by control of the host cell metabolic machinery

Some integrate into the genome

simultaneously without cell death, although cell physiology may be affected. The close association of the virus with the cell sometimes results in the integration of viral nucleic acid into the functional nucleic acid of the cell, producing a latent infection that can be transmitted intact to the progeny of the cell.

## Bacteria

Smallest living cells

Prokaryotic cell plan lacks nucleus and organelles

Bacteria are the smallest (0.1 to 10 $\mu$m) living cells. They have a cytoplasmic membrane surrounded by a cell wall; a unique interwoven polymer called peptidoglycan makes the wall rigid. The simple prokaryotic cell plan includes no mitochondria, lysosomes, endoplasmic reticulum, or other organelles. In fact, most bacteria are about the size of mitochondria. Their cytoplasm contains only ribosomes and a single, double-stranded DNA chromosome. Bacteria have no nucleus, but all the chemical elements of nucleic acid and protein synthesis are present. Although their nutritional requirements vary greatly, most bacteria are free-living, if given an appropriate energy source. Tiny metabolic factories, they divide by binary fission and can be grown in artificial culture, often in less than a day. The Archaebacteria differ radically from other bacteria in structure and metabolic processes; they live in environments humans consider hostile (eg, hot springs, high salt areas) but are not associated with disease.

## Fungi

Yeasts and molds are surrounded by cell wall

Fungi exist in either yeast or mold forms. The smallest of yeasts are similar in size to bacteria, but most are larger (2 to 12 $\mu$m) and multiply by budding. Molds form tubular extensions called hyphae, which when linked together in a branched network form the fuzzy structure seen on neglected bread. Fungi are eukaryotic, and both yeasts and molds have a rigid external cell wall composed of their own unique polymers, called glucan, mannan, and chitin. Their genome may exist in a diploid or haploid state and replicate by meiosis or simple mitosis. Most fungi are free-living and widely distributed in nature. Generally, fungi grow more slowly than bacteria, although their growth rates sometimes overlap.

## Parasites

Range from tiny amoebas to meter-long worms

Parasites are the most diverse of all microorganisms. They range from unicellular amoebas of 10 to 12 $\mu$m to multicellular tapeworms 1 meter in length. The individual cell plan is eukaryotic, but the organisms such as worms are highly differentiated and have their own organ systems. Most of the worms have a microscopic egg or larval stage, and part of their life cycle may involve multiple vertebrate and invertebrate hosts. Most parasites are free-living but some depend on combinations of animal, arthropod, or crustacean hosts for their survival.

# INFECTIOUS DISEASE

Pathogens are rare

Virulence varies greatly

Of the thousands of species of viruses, bacteria, fungi, and parasites, only a tiny portion are involved in disease of any kind. These are called pathogens. There are plant pathogens, animal pathogens, fish pathogens, as well as the subject of this book, human pathogens. Among pathogens, there are degrees of potency called virulence, which sometimes makes the dividing line between benign and virulent microorganisms difficult to draw. Many bacteria and some fungi are part of a normal flora that colonizes the skin and mucosal surfaces of the body, where most of the time they appear to do no harm. In extreme circumstances, a few of these organisms are associated with mild disease, making them low-virulence pathogens at best. Other pathogens are virtually always associated with disease of varying severity. *Yersinia pestis,* the cause of plague, causes fulminant disease and death in 50 to 75% of individuals who come in contact with it. It is highly virulent. Understanding the basis of these differences in virulence is a fundamental goal of this book. The better students of medicine understand how a pathogen causes disease, the better they will be prepared to intervene and help their patients.

For any pathogen the basic aspects of how it interacts with the host to produce disease can be expressed in terms of its epidemiology, pathogenesis, and immunity. Usually our knowledge of one or more of these topics is incomplete. It is the task of the physician to relate these topics to the clinical aspects of disease and be prepared for new developments which clarify, or in some cases, alter them. We do not know everything, and not all of what we believe we know is correct.

## EPIDEMIOLOGY

Epidemiology is the "who, what, when, and where" of infectious diseases. The power of the science of epidemiology was first demonstrated by Semmelweis, who by careful data analysis alone determined how streptococcal puerperal fever was transmitted. He even devised a means to prevent it decades before the organism itself was discovered (see Chapter 72). Since then each organism has built its own profile of vital statistics. Some agents are transmitted by the air, others by food, others by insects, and some spread by the person-to-person route. Some agents occur worldwide, and others only in certain geographic locations or ecologic circumstances. Knowing how an organism gains access to its victim and spreads are crucial to understanding the disease. It is also essential to discovering the emergence of "new" diseases, whether they are truly new (AIDS) or just undiscovered (Legionnaires' disease). Solving mysterious outbreaks or recognizing new epidemiologic patterns have usually pointed the way to the isolation of new agents.

Each agent has its own mode of spread

Epidemic spread and disease are facilitated by malnutrition, poor socioeconomic conditions, natural disasters, and hygienic inadequacy. In previous centuries, epidemics, sometimes caused by the introduction of new organisms of unusual virulence, often resulted in high morbidity and mortality. The possibility of recurrence of old pandemic infections remains, and, in the case of AIDS, we are currently witnessing a new and extended pandemic infection. Modern times and technology have introduced new wrinkles to epidemiologic spread. Intercontinental air travel has allowed diseases to leap continents even when they have very short incubation periods (cholera). The efficiency of the food industry has sometimes backfired when the distributed products are contaminated with infectious agents. The well-publicized outbreaks of hamburger-associated *Escherichia coli* O157:H7 infection are an example. The nature of massive meatpacking facilities allowed organisms from infected cattle on isolated farms to be mixed with other meat and distributed rapidly and widely. By the time outbreaks are recognized, cases of disease are widespread, and tons of meat must be recalled. In simpler times, local outbreaks from the same source would have been detected and contained more quickly.

Poor socioeconomic conditions foster infection

Modern society may facilitate spread

Of course, the most ominous and uncertain epidemiologic threat of these times is not amplification of natural transmission but the specter of unnatural, deliberate spread. Anthrax is a disease uncommonly transmitted by direct contact of animals or animal products with humans. Under natural conditions, it produces a nasty but usually not life-threatening ulcer. The inhalation of human-produced aerosols of anthrax spores could produce a lethal pneumonia on a massive scale. Smallpox is the only disease officially eradicated from the world. It took place so long ago that most of the population has never been exposed or immunized and are thus vulnerable to its reintroduction. We do not know if infectious bioterrorism will work on the scale contemplated by its perpetrators, but in the case of anthrax we do know that sophisticated systems have been designed to attempt it. We hope that we will never learn whether bioterrorism will work on a large scale.

Anthrax and smallpox are new bioterrorism threats

## PATHOGENESIS

Once a potential pathogen reaches its host, features of the organism determine whether or not disease ensues. The primary reason pathogens are so few in relation to the microbial world is that being a successful pathogen is very complicated. Multiple features, called virulence factors, are required to persist, cause disease, and escape to repeat the cycle.

Pathogenicity is multifactorial

The variations are many, but the mechanisms used by many pathogens are now being dissected at the molecular level.

The first step for any pathogen is to attach and persist at whatever site it gains access. This usually involves specialized surface molecules or structures that correspond to receptors on human cells. Because human cells were not designed to receive the microorganisms, they are usually exploiting some molecule important for essential functions of the cell. For some toxin-producing pathogens, this attachment is all they need to produce disease. For most pathogens, it just allows them to persist long enough to proceed to the next stage, invasion into or beyond the mucosal cells. For viruses, invasion of cells is essential, because they cannot replicate on their own. Invading pathogens must also be able to adapt to a new milieu. For example, the nutrients and ionic environment of the cell surface differs from that inside the cell or in the submucosa.

Pathogens have molecules that bind to host cells

Invasion requires adaptation to new environments

Persistence and even invasion do not necessarily translate immediately to disease. The invading organisms must disrupt function in some way. For some, the inflammatory response they stimulate is enough. For example, a lung alveolus filled with neutrophils responding to the presence of *Streptococcus pneumoniae* loses its ability to exchange gases. The longer a pathogen can survive in the face of the host response, the greater the compromise in host function. Most pathogens do more than this. Destruction of host cells through the production of digestive enzymes, toxins, or intracellular multiplication is among the more common mechanisms. Other pathogens operate by altering the function of a cell without injury. Cholera is caused by a bacterial toxin, which causes intestinal cells to hypersecrete water and electrolytes leading to diarrhea. Some viruses cause the insertion of molecules in the host cell membrane, which cause other host cells to attack it. The variations are diverse and fascinating.

Inflammation alone can result in injury

Cells may be destroyed or their function altered

## IMMUNITY

Although the science of immunology is beyond the scope of this book, understanding the immune response to infection (see Chapter 8) is an important part of appreciating pathogenic mechanisms. In fact, one of the most important virulence attributes any pathogen can have is an ability to evade the immune response. Some pathogens attack the immune effector cells, and others undergo changes that confound the immune response. The old observation that there seems to be no immunity to gonorrhea turns out to be an example of the latter mechanism. *Neisseria gonorrhoeae,* the causative agent of gonorrhea, undergoes antigenic variation of important surface structures so rapidly that antibodies directed against the bacteria become irrelevant.

Evading the immune response is a major feature of virulence

For each pathogen, the primary interest is whether there is natural immunity and, if so, whether it is based on humoral (antibody) or cell-mediated immunity (CMI). Humoral and CMI responses are broadly stimulated with most infections, but the specific response to a particular molecular structure is usually dominant in mediating immunity to reinfection. For example, the repeated nature of strep throat (group A streptococcus) in childhood is not due to antigenic variation as described above for gonorrhea. The antigen against which protective antibodies are directed (M protein) is stable but naturally exists in over 80 types. Each requires its own specific antibody. Knowing the molecule against which the protective immune response is directed is particularly important for devising preventive vaccines.

Antibody or cell-mediated mechanisms may be protective

# CLINICAL ASPECTS OF INFECTIOUS DISEASE

## MANIFESTATIONS

Fever, pain, and swelling are the universal signs of infection. Beyond this, the particular organs involved and the speed of the process dominate the signs and symptoms of disease. Cough, diarrhea, and mental confusion represent disruption of three different body

Body system(s) involved dictate clinical findings systems. On the basis of clinical experience, physicians have become familiar with the range of behavior of the major pathogens. However, signs and symptoms overlap considerably. Skilled physicians use this knowledge to begin a deductive process leading to a list of suspected pathogens and a strategy to make a specific diagnosis and provide patient care. Through the probability assessment, an understanding of how the diseases work is a distinct advantage in making the correct decisions.

## DIAGNOSIS

A major difference between infectious and other diseases is that the probabilities described above can be specifically resolved, often overnight. Most microorganisms can be isolated from the patient, grown in artificial culture, and identified. Others can be seen microscopically or detected by measuring the host specific immune response. Preferred modalities for diagnosis of each agent have been developed and are available in clinic, hospital, and public health laboratories all over the world. Empiric diagnosis made on the basis of clinical findings can be confirmed and the treatment plan modified accordingly. The new molecular methods, which detect molecular structures or genes of the agent, are not yet practical for most infectious diseases.

Disease-causing microbes can be grown and identified

## TREATMENT

Over the past 60 years, therapeutic tools of remarkable potency and specificity have become available for the treatment of bacterial infections. These include all the antibiotics and an array of synthetic chemicals that kill or inhibit the infecting organism but have minimal or acceptable toxicity for the host. Antibacterial agents exploit the structural and metabolic differences between bacterial and eukaryotic cells to provide the selectivity necessary for good antimicrobial therapy. Penicillin, for example, interferes with the synthesis of the bacterial cell wall, a structure that has no analog in human cells. There are fewer antifungal and antiprotozoal agents because the eukaryotic cells of the host and those of the parasite have close metabolic and structural similarities. Nevertheless, hosts and parasites do have some significant differences, and effective therapeutic agents have been discovered or developed to exploit them.

Antibiotics are directed at structures of bacteria not present in host

Specific therapeutic attack on viral disease has posed more complex problems, because of the intimate involvement of viral replication with the metabolic and replicative activities of the cell. Thus, most substances that inhibit viral replication have unacceptable toxicity to host cells. However, recent advances in molecular virology have identified specific viral targets that can be attacked. Scientists have developed some successful antiviral agents, including agents that interfere with the liberation of viral nucleic acid from its protective protein coat or with the processes of viral nucleic acid synthesis and replication. The successful development of new agents for human immunodeficiency virus has involved targeting enzymes coded by the virus genome.

Antivirals target unique virus-coded enzymes

The success of the "antibiotic era" has been clouded by the development of resistance by the organisms. The mechanisms involved are varied but most often involve a mutational alteration in the enzyme, ribosome site, or other target against which the antimicrobial is directed. In some instances, the organisms acquire new enzymes or block entry of the antimicrobic to the cell. Many bacteria produce enzymes which directly inactivate antibiotics. To make the situation worse, the genes involved are readily spread by promiscuous genetic mechanisms. New agents that are initially effective against resistant strains have been developed, but resistance by new mechanisms usually follows. The battle is by no means lost but has become a never-ending policing action.

Resistance complicates therapy

Mechanisms include mutation and inactivation

## PREVENTION

The ultimate outcome with any disease is its prevention. In the case of infectious diseases, this has involved public health measures and immunization. The public health measures depend on knowledge of transmission mechanisms and interfering with them. Water disinfection, food preparation, insect control, handwashing, and a myriad of other

Public health and immunization are primary preventive measures

measures prevent humans from coming in contact with infections agents. Immunization relies on knowledge of immune mechanisms and designing vaccines that stimulate protective immunity.

Attenuated strains stimulate immunity

Live vaccines can cause disease

Immunization follows two major strategies, live and inactivated vaccines. The former uses live but attenuated organisms that have been modified so they do not produce disease but still stimulate a protective immune response. Such vaccines have been effective but carry the risk that the vaccine strain itself may cause disease. This event has been observed with the live oral polio vaccine. Although this rarely occurs, it has caused a shift back to the original Salk inactivated vaccine. This issue has reemerged with a debate over strategies for the use of smallpox immunization to protect against bioterrorism. This vaccine uses vaccinia virus, a cousin of smallpox, and its potential to produce disease on its own has been recognized since its original use by Jenner in 1798. Serious disease would be expected primarily in immunocompromised individuals, who represent a significantly larger part of the population (eg, from cancer chemotherapy, AIDS) than when smallpox immunization was stopped in the 1970s. Could immunization cause more disease than it prevents? The question is difficult to answer.

Purified components are safe vaccines

The safest immunization strategy is the use of organisms that have been killed or, better yet, killed and purified to contain only the immunizing component. This approach requires much better knowledge of pathogenesis and immune mechanisms. Vaccines for meningitis use only the polysaccharide capsule of the bacterium, and vaccines for diphtheria and tetanus use only a formalin-inactivated protein toxin. Pertussis (whooping cough) immunization has undergone a transition in this regard. The original killed whole-cell vaccine was effective but caused a significant frequency of side effects. A purified vaccine containing pertussis toxin and a few surface components has reduced side effects while retaining efficacy.

Vaccines can be genetically engineered

The newest approaches for vaccines require neither live organisms nor killed, purified ones. As the entire genomes of more and more pathogens are being reported, an entirely genetic strategy is emerging. Armed with knowledge of molecular pathogenesis and immunity and the tools of genomics and proteomics, scientists can now synthesize an immunogenic protein without ever growing the organism itself. Such an idea would have astonished even the great microbiologists of the past two centuries.

## SUMMARY

Infectious diseases remain as important and fascinating as ever. Where else do we find the emergence of new diseases, together with improved understanding of the old ones? At a time when the revolution in molecular biology and genetics has brought us to the threshold of new and novel means of infection control, the perpetrators of bioterrorism threaten us with diseases we have already conquered. Meeting this challenge requires a secure knowledge of the pathogenic organisms and how they produce disease, as well as an understanding of the clinical aspects of those diseases. In the collective judgment of the authors, this book presents the principles and facts required for students of medicine to understand the most important infectious diseases.

PART I

# The Bacterial Cell

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CHAPTER 2

# Bacterial Structures

FREDERICK C. NEIDHARDT

This chapter examines the special structural, architectural and chemical features of the prokaryotic (bacterial) cell that contribute to the ubiquity of this large group of organisms and their ability to cause disease in humans. Discussion focuses particularly on the characteristics that distinguish bacteria from the more familiar cells of eukaryotes, which therefore offer the opportunity for medical interventions.

## GENERAL MORPHOLOGY, BODY PLAN, AND COMPOSITION

The bacterial cell that is seen today is closer in form to the primordial cells of our planet than is any animal or plant cell. This similarity is misleading, however, because bacteria are the product of close to 3 billion years of natural selection and have emerged as immensely diverse and successful organisms that colonize almost all parts of the world and its other inhabitants. Because bacteria have remained microscopic, it can be concluded that very small size per se is not a disadvantage in nature but rather provides unique opportunities for survival and reproduction. Thus, the first major principle to help us understand bacteria is their small size.

Bacteria are highly successful colonizers

Bacteria are by far the smallest living cells, and some are considered to have the minimum possible size for an independently reproducing organism. Individuals of different bacterial species that colonize or infect humans range from 0.1 to 10 $\mu$m (1 $\mu$m = $10^{-6}$ m) in their largest dimension. Most spherical bacteria have diameters of 0.5 to 2 $\mu$m, and rod-shaped cells are generally 0.2 to 2 $\mu$m wide and 1 to 10 $\mu$m long. At the lower end of the scale, some bacteria (rickettsias, chlamydia, and mycoplasmas) overlap with the largest viruses (the poxviruses), and at the upper end, some rod-shaped bacteria have a length equal to the diameter of some eukaryotic cells (Fig 2–1). As a shorthand approximation, bacteria are sole possessors of the 1-$\mu$m size.

Most bacteria are in the range of 1–10 $\mu$m

A wealth of structural detail cannot be discerned in bacteria even with the best of light microscopes because of their small size and because they are nearly colorless and transparent and have a refractive index similar to that of the surrounding liquid. However, shape can easily be discerned with appropriate microscopic techniques, and distinctive shapes are characteristic of broad groupings of bacteria (Fig 2–2). The major forms that can be recognized are spheres, rods, bent or curved rods, and spirals. Spherical or oval bacteria are called **cocci** (singular: **coccus**). Rods are called **bacilli** (singular: **bacillus**). Very short rods that can sometimes almost be mistaken for cocci are called **coccobacilli.** Some rod-shaped bacteria have tapered ends and are therefore termed **fusiform,** whereas others are characteristically club-shaped and may be curved or bent. Spiral-shaped bacteria are called **spirilla** if the cells are rigid and **spirochetes** if they are more flexible and undulating.

Bacteria exhibit a variety of shapes and cell arrangements

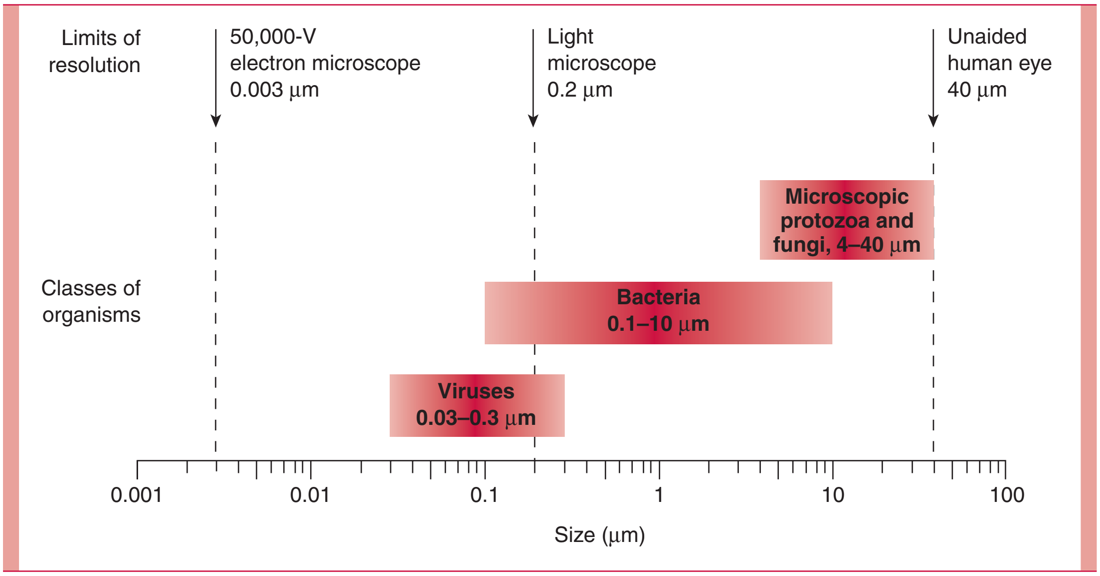


FIGURE 2–1
Relative sizes of microorganisms.

In addition to shape, distinctive arrangements of groups of cells can readily be observed for some bacterial genera (Fig 2–3). The reason one can speak of arrangements of unicellular organisms is that there is a tendency, varying with different genera, for newly divided cells to stick together. The nature of the aggregates formed depends on the degree of stickiness (which can vary with growth conditions) and on the plane of successive cell divisions. Among the cocci, pairs (**diplococci**), chains (**streptococci**), and irregular clusters (**staphylococci**) are found. A few genera of bacteria were named for their distinctive shape or cell arrangement. There are many thousands of species of bacteria, however, so it should be clear that the shape and arrangement of cells cannot be taken far in identifying the particular organism in a given sample or culture. A further caution for medical microbiologists is the tendency of some bacteria to take on altered shapes and arrangements when in contact with various antimicrobics.

Some antimicrobics affect cell morphology

Whatever the overall shape of the cell, the 1-μm size could not accommodate the familiar eukaryotic cell plan. There is insufficient room for mitochondria, nucleus, Golgi apparatus, lysosomes, endoplasmic reticulum, and the like in a cell that is itself only as large as an average mitochondrion. The design of the bacterial cell must thus differ fundamentally from that of other cells. This is precisely the case, and the unique design is designated **prokaryotic.**

Prokaryotic cell design is unique

A generalized bacterial cell is shown in Figure 2–4. The major structures of the cell belong either to the multilayered **envelope** and its **appendages** or to the interior core consisting of the **nucleoid** (or nuclear body) and the **cytosol** (called thus rather than cytoplasm because there is no nucleus; the cytosol is not separated from the genetic material). In contrast to the alien nature of this body plan, the general chemical nature of the bacterial cell is more familiar to a eukaryotic cell biologist. Greater than 90% of its dry mass consists of five macromolecular-like substances similar to those found in eukaryotes: proteins (about 55% of the dry mass); RNA, consisting of the familiar messenger (mRNA), transfer (tRNA), and

Major structures form the envelope, appendages, cytosol, and nucleoid

Chemical nature is similar to eukaryotic cells but with some unique components

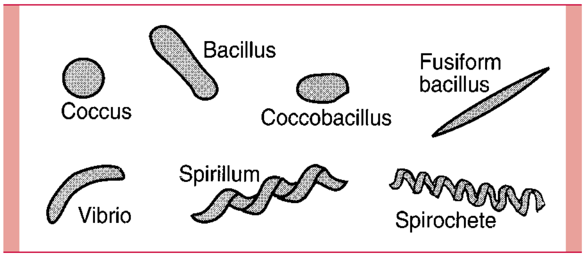


FIGURE 2–2
Shapes of some different bacteria.

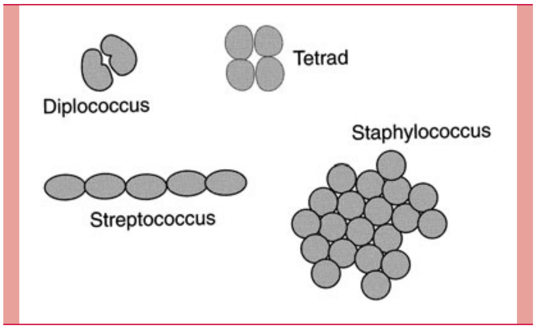


FIGURE 2–3
Arrangement of spherical bacterial cells.

ribosomal (rRNA) types (about 20%); DNA (about 3%); carbohydrate (about 5%); and phospholipid (about 6%). In addition, there are a few macromolecules unique to prokaryotes; a peptidoglycan called **murein** is found in all walled bacteria, and a few other unique molecules (lipopolysaccharide and teichoic acids) are found in specific groups of bacteria.

As we shall see, small size and extraordinarily simple design help explain the success of bacteria in nature. Small size facilitates rapid exchange of nutrients and metabolic byproducts with the environment, whereas simplicity of design facilitates macromolecular synthesis, assembly of cell structures, and formation of new cells by division. Both smallness and simplicity contribute to a distinctive functional property of bacteria—their ability to grow at least an order of magnitude faster than eukaryotic cells. However, at the molecular level, bacteria are far from simple, and it is necessary to learn something of their complexity at this level to understand the ability of some of them to colonize humans or to cause disease.

Small size and simple design facilitate rapid growth

## ENVELOPE AND APPENDAGES

As a first approximation, bacteria can be said to have a plain interior and a fancy exterior. The cell core, consisting solely of nucleoid and cytosol, is incredibly simple and almost structureless compared with the interior of a eukaryotic cell. It fits the notion that simplicity facilitates rapid growth. The envelope, on the other hand, is an exceedingly baroque part of the cell, consisting of structures of great complexity that vary in detail among the different major groups of bacteria. This can be readily understood by appreciating three important

Complex structure of cell envelope fits its multiple functions

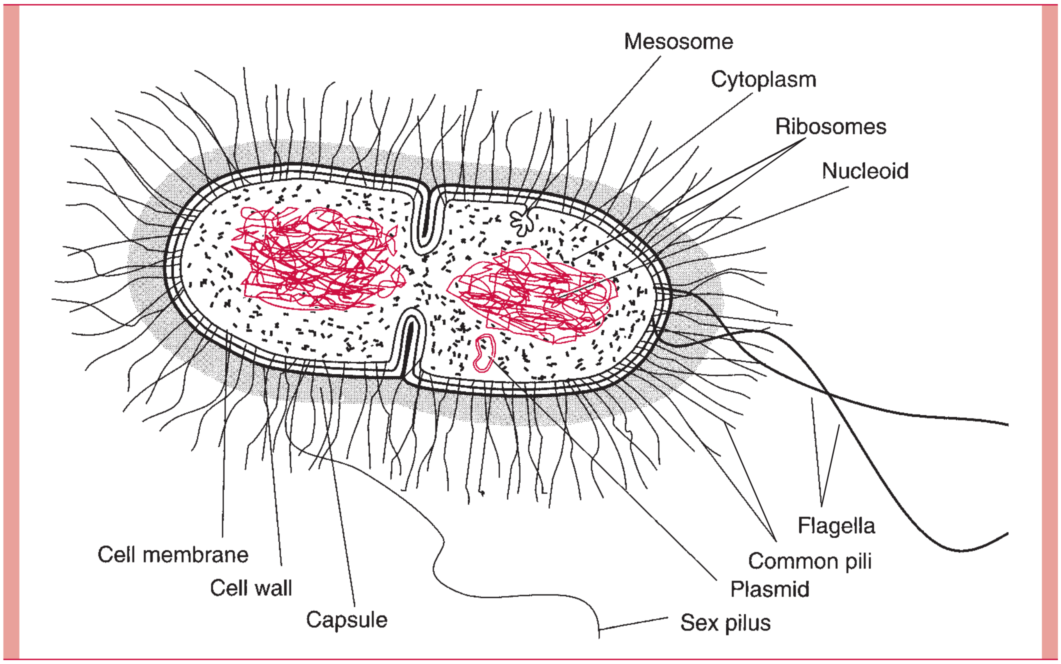


FIGURE 2–4
Schematic of structures of a dividing bacterium.

TABLE 2–1

**Components of Bacterial Cells**

| | | Distribution[a] | | |
|---|---|---|---|---|
| Structure | Composition | Gram-Negative Cell | Gram-Positive Cell | Mollicutes (Mycoplasmas) |
| **Envelope** | | | | |
| Capsule (slime layer) | Polysaccharide or polypeptide | + or − | + or − | − |
| Wall | | + | + | − |
| Outer membrane | Proteins, phospholipids, and lipopolysaccharide | + | − | − |
| Peptidoglycan layer | Murein (+ teichoate in Gram-positive cells) | + | + | − |
| Periplasm | Proteins and oligosaccharides in solution | + | − | − |
| Cell membrane | Proteins, phospholipids | + | + | + |
| **Appendages** | | | | |
| Pili (fimbriae) | Protein (pilin) | + or − | + or − | − |
| Flagella | Proteins (flagellin plus others) | + or − | + or − | − |
| **Core** | | | | |
| Cytosol | Polyribosomes, proteins, carbohydrates (glycogen) | + | + | + |
| Nucleoid | DNA with associated RNA and proteins | + | + | + |
| Plasmids | DNA | + or − | + or − | + or − |
| **Endospore** | | | | |
| All cell components plus dipicolinate and special envelope components | | − | + or − | − |

[a] "+" indicates the structure is invariably present, "−" indicates it is invariably absent, and "+ or −" indicates that the structure is present is some species or strains and absent in others.

principles of bacterial functional anatomy: (1) the envelope is responsible for many cellular processes that are the province of the internal organelles of eukaryotic cells, (2) the envelope is the primary site of functions that protect the bacterial cell against chemical and biological threats in its environment, and (3) the envelope and certain appendages make possible the colonization of surfaces by bacteria. Not surprisingly, therefore, more than one fifth of the specific proteins of well-studied bacteria are located in the envelope. Differences in envelope structure and composition (Table 2–1) are the basis of the assignment, described next, of all eubacterial species to one of three major groups: (1) Gram-negative bacteria; (2) Gram-positive bacteria; and (3) wall-less bacteria, including the mollicutes (mycoplasmas) and chlamydia. Figure 2–5 shows schematically these major differences.

## Capsule

Hydrophilic capsule gives colonies a smooth appearance, unlike nonmucoid rough variants

Many bacterial cells surround themselves with one or another kind of hydrophilic gel. This layer is often quite thick; commonly it is thicker than the diameter of the cell. Because it is transparent and not readily stained, this layer is usually not appreciated unless made visible by its ability to exclude particulate material, such as India ink. If the

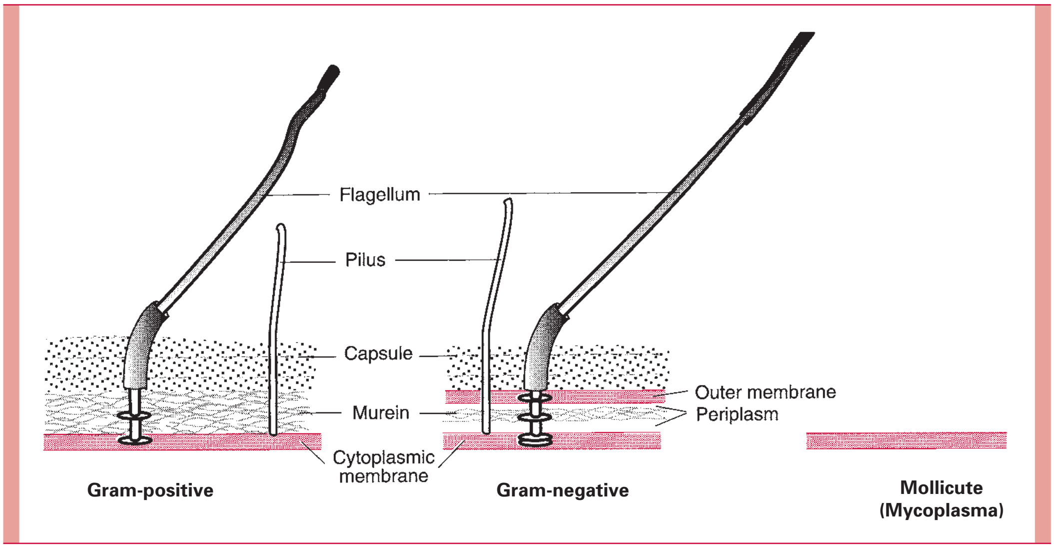


FIGURE 2–5
Schematic representation comparing the envelopes of Gram-positive bacteria, Gram-negative bacteria, and mollicutes.

material forms a reasonably discrete layer, it is called a **capsule;** if it is amorphous in appearance, it is referred to as a **slime layer.** Almost all bacterial species can make such material to some degree. Most capsules or slime layers are polysaccharides made of single or multiple types of sugar residues; some are simple (though unusual) polypeptides, such as the polymer of D-glutamic acid, which forms the capsule of *Bacillus anthracis,* the causative agent of anthrax (see Chapter 18); a few are proteins. When cultured on solid media (see Chapter 15), encapsulated bacteria give rise to smooth, often mucus-like colonies, but unencapsulated variants are common, particularly with long-term laboratory cultivation. Their colonies are nonmucoid and described as "rough."

Capsules can protect bacteria. Within animal and human hosts capsules impede ingestion by leukocytes. *Streptococcus pneumoniae,* the causative agent of pneumococcal pneumonia, in large measure owes its virulence to the ability of its copious polysaccharide capsule to interfere with opsonophagocytosis (see Chapter 17). The pneumococcal polysaccharide, as is the case with most capsular material, is antigenic (see Chapter 8), and when specific antibody attaches to it, phagocytosis can occur. A mouse–pneumococcus experimental model is instructive. Unencapsulated pneumococci are tolerated by mice; however, a single encapsulated cell injected intraperitoneally will kill a mouse unless the mouse has been immunized with capsular material of the specific antigenic type of the infecting pneumococcus, in which case it is protected. More than 80 capsular serotypes of this organism are known, reflecting a diverse genetic capacity of the species to produce capsular polysaccharides of differing chemical structure.

Antiphagocytic effect of some capsules is major virulence determinant

Protection against phagocytosis is only part of the much broader function of bacterial capsules in nature, which is to aid colonization, primarily by assisting the cell to attach to surfaces. For example, the ability of *Streptococcus mutans* and *Streptococcus salivarius* cells to adhere to the surface of teeth is in large measure a function of the polysaccharide capsules of these oral bacteria (see Chapter 62).

Some capsules promote adherence and colonization

Capsules do not contribute to growth and multiplication and are not essential for cell survival in artificial culture. Capsule synthesis is greatly dependent on growth conditions. For example, the capsule made by the caries-producing *S. mutans* consists of a dextran–carbohydrate polymer made only in the presence of sucrose.

Capsule synthesis depends on growth conditions

## Cell Wall

Internal to the capsule (if one exists) but still outside the cell proper, a rigid **cell wall** surrounds all eubacterial cells except wall-less bacteria such as the mollicutes (mycoplasmas) and *Chlamydia*. The structure and function of the bacterial wall is so distinctive as

Unique wall structure prevents osmotic lysis, determines shape, protects against toxins and phagocytosis, and helps in colonization

to constitute a hallmark of the prokaryotes; nothing like it is found elsewhere. Unlike the capsule, which is dispensable for survival outside the body of the host, the wall has vital functions in all environments. It protects the cell from mechanical disruption and from bursting caused by the turgor pressure resulting from the hypertonicity of the cell interior relative to the environment. The wall provides a barrier against certain toxic chemical and biological agents. In some bacterial species, such as *Streptococcus* (see Chapter 17), it provides a protection from phagocytosis and helps in the binding to eukaryotic cell hosts. Its form is responsible for the shape of the cell.

Gram stain distinguishes two major envelope structures

Bacterial evolution has led to two major solutions to the challenge of constructing a wall that can protect a minute, fragile cell from chemical and physical assault while still permitting the rapid exchange of nutrients and metabolic byproducts required by rapid growth. Long before these solutions were understood in ultrastructural terms, it was recognized that bacteria could be divided into two groups depending on their reaction to a particular staining procedure devised a century ago by the Danish microbiologist Hans Christian Gram. This procedure, the Gram stain, is described in detail in Chapter 15. It depends on the differential ability of ethanol or ethanol–acetone mixtures to extract iodine–crystal violet complexes from bacterial cells. These complexes are readily extracted from one group of bacteria, termed **Gram-negative,** which can be subsequently stained red with an appropriate counterstain. They are retained by the other, termed **Gram-positive,** which are thus stained violet by the retained crystal violet. The positive or negative Gram stain response of a cell reflects which of the two types of wall it possesses.

A few pathogens are not usefully distinguished by Gram stain

Virtually all of the eubacteria with walls can be assigned a Gram response. However, the few exceptions include some medically important organisms. For example, the mycobacteria (eg, *Mycobacterium tuberculosis,* the causative agent of tuberculosis) are Gram positive on the basis of their wall structure but fail to stain because of interference by special lipids present in their walls. Most spirochetes, including *Treponema pallidum* (the causative agent of syphilis), although Gram negative by structure, are too thin to be resolved in the light microscope when stained by simple stains.

Cells can lose Gram-positive trait

Bacteria without walls, whether natural forms (the mollicutes or mycoplasmas) or artificial products of procedures that remove the wall, exhibit a Gram-negative staining response. Furthermore, some bacteria that are Gram positive on the basis of wall structure and staining response may lose this property and appear Gram negative if they have been held under nongrowing conditions. These examples emphasize that being Gram positive is a distinct property that can be temporarily lost because it depends on the integrity of the cell wall; on the other hand, a Gram-negative bacterial cell does not have a staining property to lose.

## *Gram-Positive Cell Wall*

Major components of Gram-positive walls are peptidoglycan and teichoic acid

The Gram-positive cell wall contains two major components, peptidoglycan and teichoic acids, plus additional carbohydrates and proteins, depending on the species. A generalized scheme illustrating the arrangement of these components is shown in Figure 2–6.

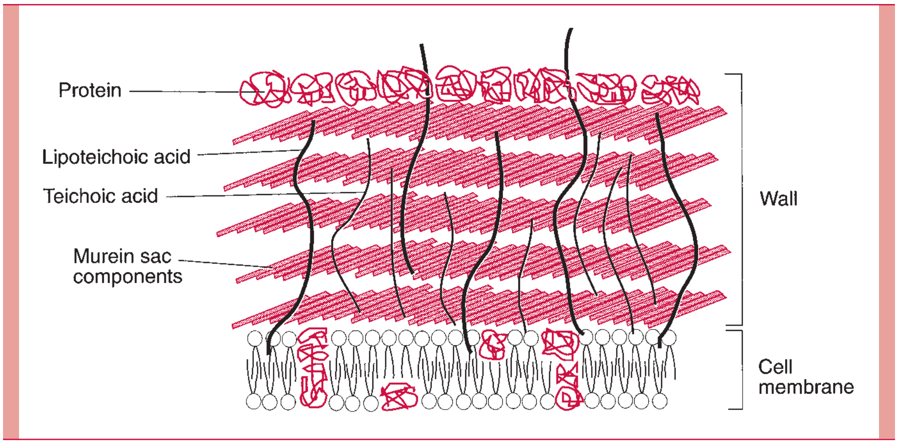


FIGURE 2–6
Schematic representation of the wall of Gram-positive bacteria.

The chief component is **murein,** a peptidoglycan, which is found nowhere except in prokaryotes. Murein consists of a linear glycan chain of two alternating sugars, *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM), in 1:4 linkages (Fig 2–7). Each muramic acid residue bears a tetrapeptide of alternating L- and D-amino acids. Adjacent glycan chains are cross-linked into sheets by peptide bonds between the third amino acid of one tetrapeptide and the terminal D-alanine of another. The same cross-links between other tetrapeptides connect the sheets to form a three-dimensional, rigid matrix. The cross-links involve perhaps one third of the tetrapeptides and may be direct or may include a peptide bridge, as, for example, a pentaglycine bridge in *Staphylococcus aureus*. The cross-linking extends around the cell, producing a scaffold-like giant molecule, termed the **murein sac,** or **sacculus.** Murein is much the same in all bacteria, except that there is diversity in the nature and frequency of the cross-linking bridge and in the nature of the amino acids at positions 2 and 3 of the tetrapeptide.

Murein comprises linear glycan chains of alternating NAG and NAM cross-linked in three dimensions by peptide chains

Scaffold-like murein sac surrounds cell

The murein sac derives its great mechanical strength from the fact that it is a single, covalently bonded structure; other features contributing strength are the $\beta$-1,4 bonds of the polysaccharide backbone, the alternation of D- and L-amino acids in the tetrapeptide, and extensive internal hydrogen bonding. Biological stability is contributed by components of murein that are not widely distributed in the biological world or in fact are unique to murein. These include muramic acid, D-amino acids, and diaminopimelic acid (an amino acid found in the tetrapeptide of some species). Most enzymes found in mammalian hosts and other biological systems do not degrade peptidoglycan; one important exception is lysozyme, the hydrolase present in tears and other secretions, which cleaves the $\beta$-1,4 glycosidic bond between muramic acid and glucosamine residues (see Fig 2–7). On the other hand, bacteria themselves are rich in hydrolases that degrade peptidoglycan, because the murein sac must be constantly expanded by insertion of new chains as the cell grows and forms a cross-wall preparatory to cell division. As we shall learn, disruption of the fine control that bacteria exert over the activity of these potentially

Rare or unique components of murein provide resistance to most mammalian enzymes

Bacterial enzymes insert new murein chains during growth and provide targets for antimicrobics

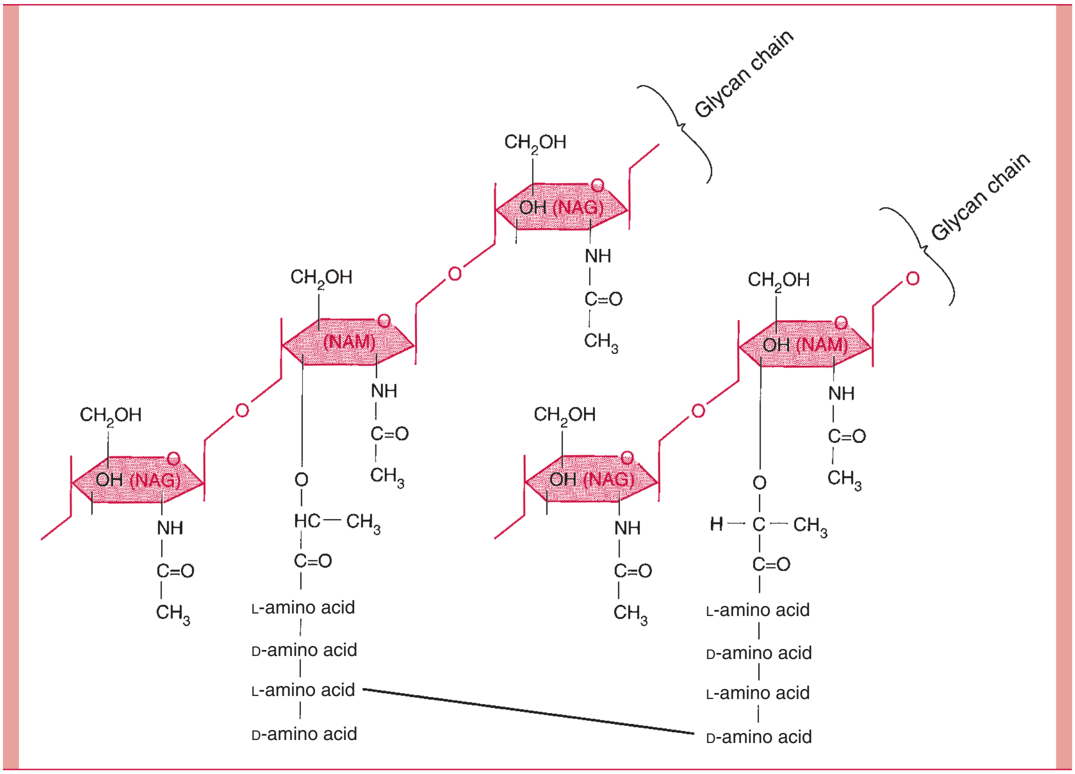


FIGURE 2–7
Schematic representation of the peptidoglycan murein. NAG, *N*-acetylglucosamine; NAM, *N*-acetylmuramic acid.

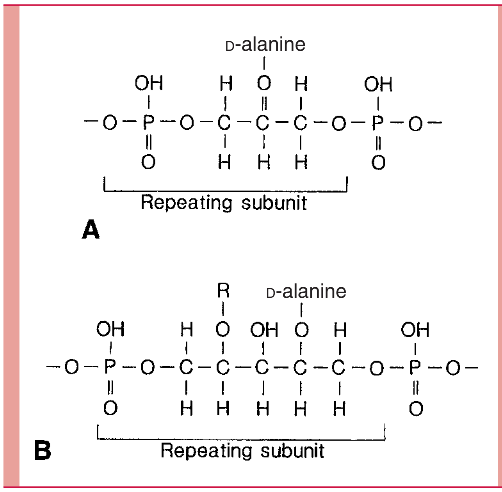


FIGURE 2–8
Schematic reproduction of teichoic acids. **A**. Glycerol teichoic acid. **B**. A ribitol teichoic acid in which R may be glucose or succinate in different species.

lethal enzymes is the means by which a large number of antibiotics and other chemotherapeutic compounds work (see Chapter 13).

Loss of cell wall leads to lysis in hypotonic media or production of protoplasts in isotonic media

The role of the murein component of the cell wall in conferring osmotic resistance and shape on the cell is easily demonstrated by removing or destroying it. Treatment of a Gram-positive cell with penicillin (which blocks formation of the tetrapeptide cross-links and activates the cell's own murein hydrolases) or with lysozyme (which directly hydrolyzes the glycan chains) destroys the murein sac, and the wall is lost. Prompt lysis of the cell ensues. If the cell is protected from lysis by suspension in a medium approximately isotonic with the cell interior, such as 20% sucrose, the cell becomes round and forms a sphere called a **protoplast.** Some protoplasts can grow, and their formation from classic bacteria within patients treated with penicillin-type antibiotics (L-forms) has been postulated to account for some persistent infections. Superficially, protoplasts resemble the mollicutes (mycoplasmas) that are naturally wall-less bacteria.

Teichoic and lipoteichoic acids promote adhesion and anchor wall to membrane

Different teichoic acids occur in different Gram-positive genera

A second component of the Gram-positive cell wall is a **teichoic** acid. These compounds are polymers of either glycerol phosphate or ribitol phosphate, with various sugars, amino sugars, and amino acids as substituents (Fig 2–8). The lengths of the chain and the nature and location of the substituents vary from species to species and sometimes between strains within a species. Up to 50% of the wall may be teichoic acid, some of which is covalently linked to occasional NAM residues of the murein. Of the teichoic acids made of polyglycerol phosphate, much is linked not to the wall but to a glycolipid in the underlying cell membrane. This type of teichoic acid is called **lipoteichoic acid** and seems to play a role in anchoring the wall to the cell membrane and as an epithelial cell adhesin. Teichoic acids are found only in Gram-positive cells and constitute major antigenic determinants of their cell surface individuality. For example, *S. aureus* polysaccharide A is a teichoic acid and *Enterococcus faecalis* group D carbohydrate is a lipoteichoic acid.

Other cell wall components offer protection and promote colonization

Beside the major wall components—murein and teichoic acids—Gram-positive walls usually have lesser amounts of other molecules. Some are polysaccharides, such as the group-specific antigens of streptococci; others are proteins, such as the M protein of group A streptococci. The detailed arrangement of the various antigens in some of the more complex Gram-positive walls is still being worked out, but minor components are thought to protect the peptidoglycan layer from the action of such agents as lysozyme. Some protein components, called **adhesins,** of the cell wall promote colonization by sticking the bacteria to the surfaces of host cells (see Chapter 10).

## *Gram-Negative Cell Wall*

Thin murein sac is imbedded in periplasmic murein gel

The second kind of cell wall found in bacteria, the Gram-negative cell wall, is depicted in Figure 2–9. Except for the presence of murein, there is little chemical resemblance to cell walls of Gram-positive bacteria, and the architecture is fundamentally different. In

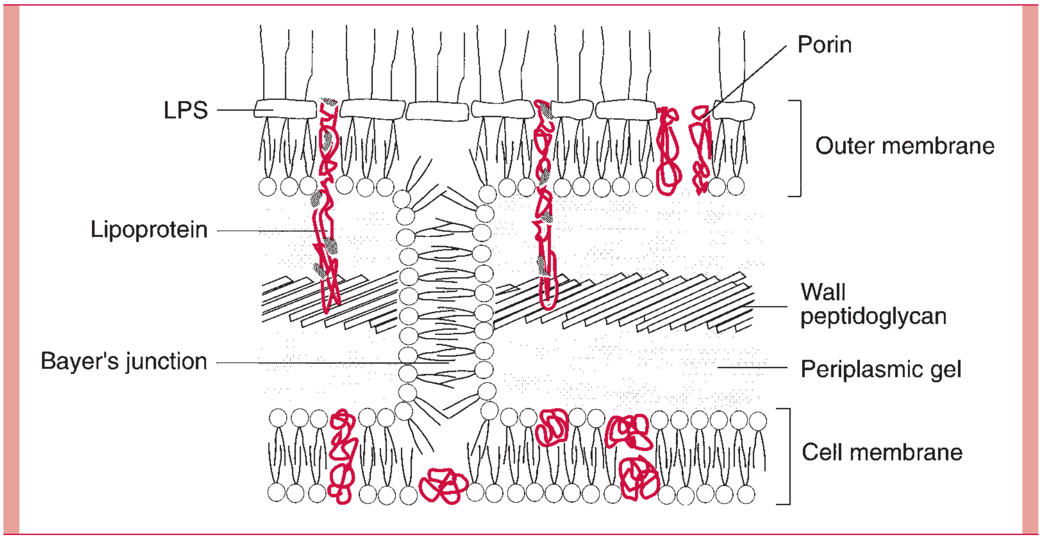


FIGURE 2–9
Schematic representation of wall of Gram-negative bacteria. LPS, lipopolysaccharide with endotoxic properties.

Gram-negative cells, the amount of murein has been greatly reduced, with some of it forming a single-layered sheet around the cell and the rest forming a gel-like substance, the **periplasmic gel,** with little cross-linking. External to this **periplasm** is an elaborate outer membrane.

Historically, the cell wall was regarded as the structure external to the cell membrane (excluding the capsule), and for Gram-positive bacteria this conception is certainly appropriate. Examination of Figures 2–5 and 2–9 shows the dilemma in applying the same term to the Gram-negative envelope. There is some reason to apply the same definition used for the Gram-positive situation, in which case the cell wall of Gram-negative bacteria consists of periplasm with its murein sac plus the outer membrane. This convention is used in Table 2–1 and in the text of this chapter. An alternative convention is to consider that the cell wall of Gram-negative bacteria is simply the structure chemically most like the Gram-positive wall, namely, the thin murein sac, with perhaps its attendant periplasmic gel. The student will quickly realize the underlying truth that **cell wall** is not a very satisfying term. Some microbiologists use cell envelope and envelope layers and avoid using the term cell wall altogether for Gram-negative bacteria.

Gram-negative wall is murein sac plus outer membrane

Earlier electron micrographs had suggested that the small amount of murein in Gram-negative cells, such as *Escherichia coli,* formed a single sheet around the cell, and that this murein sac was floating in a space, the periplasmic space, containing a fairly concentrated solution of proteins and oligosaccharides. Recent evidence modifies this picture and indicates that the “space” is a gel formed by murein peptidoglycan chains with little or no cross-linking.

Whatever its precise nature, the periplasm contains a murein sac, with a unit peptidoglycan structure quite similar to that in Gram-positive cells. Despite its reduced extent in the Gram-negative wall, the murein sac still is responsible for the shape of the cell and is vital for its integrity. As in the case of Gram-positive cells, removing or damaging the peptidoglycan layer leads to cell lysis. If the cells are protected from osmotic lysis during lysozyme or penicillin treatment, they assume a spherical shape. Because such spheres cannot be totally stripped of wall material, they are called **spheroplasts,** in contrast to the protoplasts formed from Gram-positive cells. Spheroplasts of some species can multiply.

Murein sac is responsible for shape and integrity; removal results in spheroplasts

The proteins in solution in the periplasm consist of enzymes with hydrolytic functions (such as alkaline phosphatase), sometimes antibiotic-inactivating enzymes, and various binding proteins with roles in chemotaxis and in the active transport of solutes into the cell (see Chapter 3). Oligosaccharides secreted into the periplasm in response to external conditions serve to create an osmotic pressure buffer for the cell.

Periplasmic proteins have transport, chemotactic, and hydrolytic roles

Gram-negative outer membrane is phospholipoprotein bilayer, of which the outer leaflet is LPS endotoxin

The periplasm is an intermembrane structure, lying between the cell membrane (discussed later) and a special membrane unique to Gram-negative cells, the **outer membrane.** This has an overall structure similar to most biological membranes with two opposing phospholipid–protein leaflets. However, in terms of its composition, the outer membrane is unique in all biology. Its inner leaflet consists of ordinary phospholipids, but these are replaced in the outer leaflet by a special molecule called **lipopolysaccharide** (LPS), which is extremely toxic to humans and other animals, and is called an **endotoxin.** Even in minute amounts, such as the amount released to the circulation during the course of a Gram-negative infection, this substance can produce a fever and shock syndrome called **Gram-negative shock,** or **endotoxic shock.**

Lipid A is toxic moiety of LPS; polysaccharides are antigenic determinants

LPS consists of a toxic **lipid A** (a phospholipid containing glucosamine rather than glycerol), a **core polysaccharide** (containing some unusual carbohydrate residues and fairly constant in structure among related species of bacteria), and **O antigen polysaccharide side chains** (Fig 2–10). The last component constitutes the major surface antigen of Gram-negative cells (which, it is recalled, lack teichoic acids).

Impermeability of outer membrane is overcome by active transport and porins

The presence of LPS in the outer leaflet of the outer membrane results in the covering of Gram-negative cells by a wall that should block the passage of virtually every organic molecule into the cell. Hydrophobic molecules (such as some antibiotics) would be blocked by the hydrophilic layer of O antigen; hydrophilic solutes, including most nutrients, such as sugars and amino acids, would face the barrier created by the

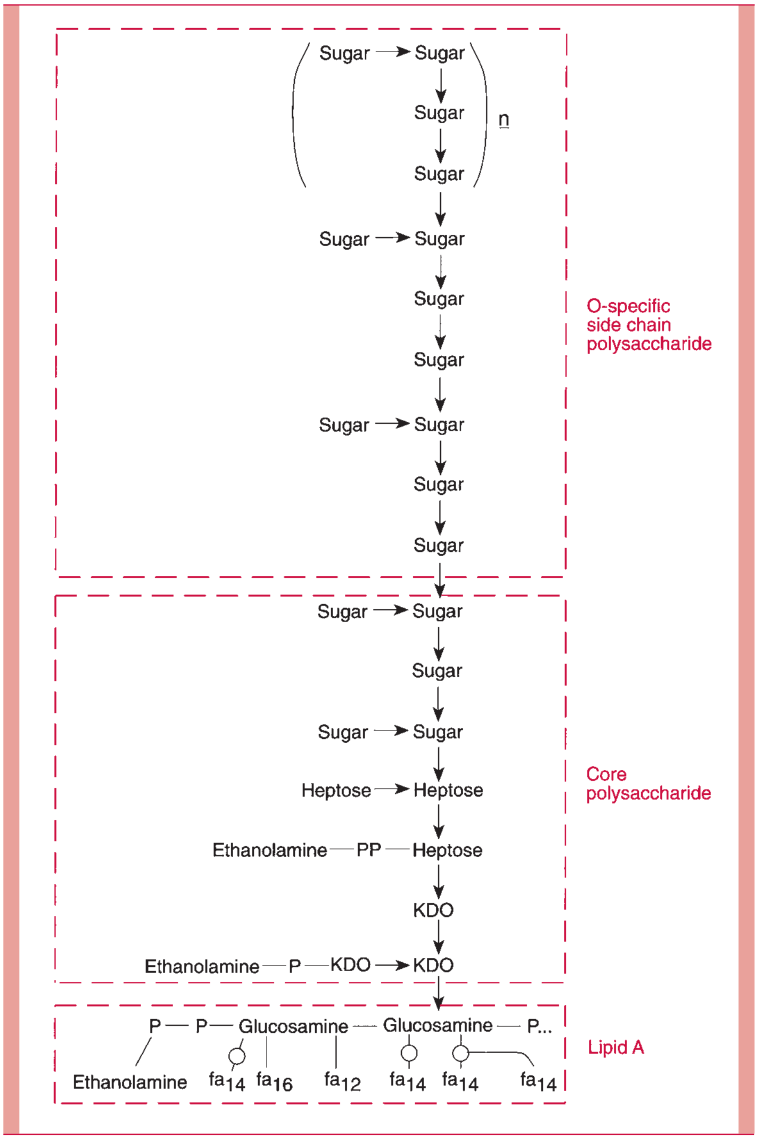


FIGURE 2–10
Schematic representation of lipopolysaccharide. The O-specific side chain is highly variable among species and subspecies and is a major determinant of antigenetic specificity. fa, fatty acid; KDO, ketodeoxyoctanate.

lipid portion of the outer membrane. Clearly this is a trade-off that cannot be made; the Gram-negative cell, for whatever benefit is afforded by possessing a wall with an outer membrane, must make provision for the rapid entry of nutrients. Active transport (described in Chapter 3) is part of the solution, and a particular structural feature of the outer membrane contributes another part. Special proteins, called **porins** or **matrix proteins,** form pores through the outer membrane that make it possible for hydrophilic solute molecules of molecular weight less than about 800 to diffuse through it and into the periplasm.

The outer membrane does not contain the variety of proteins present in the cell membrane, but those that are present are quite abundant. In addition to the porins, there is a protein called **Braun's lipoprotein** or **murein lipoprotein,** which is probably the most abundant outer membrane protein in Gram-negative cells, such as *E. coli.* This protein is covalently attached at its amino end to a lipid embedded in the outer membrane. About one third of these lipoprotein molecules are covalently attached at their carboxyl end to the third amino acid in the murein tetrapeptide. It is believed that this forms the major attachment of the murein layer to the outer membrane of the wall in *E. coli* (see Fig 2–9).

Murein lipoprotein is abundant component that attaches murein sac to outer membrane

The innermost leaflet may well be contiguous in places with the outermost leaflet of the cell membrane (see Fig 2–9), because, at least under the electron microscope, preparations of the outer membrane and the cell membrane can be seen to adhere to each other at **zones of adhesion** (also called **Bayer's junctions**). Other zones of adhesion girding the whole circumference of *E. coli* and related species have been postulated. Because these annular rings tend to form about the cell division septum, they have been called **periseptal annuli.** Their existence is still being examined.

Outer and inner membranes may be adherent in places

In evolving a cell wall containing an outer membrane, Gram-negative bacteria have succeeded in (1) creating the periplasm, which holds digestive and protective enzymes and proteins important in transport and chemotaxis; (2) presenting an outer surface with strong negative charge, which is important in evading phagocytosis and the action of complement; and (3) providing a permeability barrier against such dangerous molecules as host lysozyme, $\beta$-lysin, bile salts, digestive enzymes, and many antibiotics.

Outer membrane has many functions

## Cell Membrane

Generally the cell membrane of bacteria is similar to the familiar bileaflet membrane, containing phospholipids and proteins, that is found throughout the living world. However, there are important differences. The bacterial cell membrane is exceptionally rich in proteins (up to 70% of its weight) and does not (except in the case of mycoplasmas) contain sterols. The bacterial chromosome is attached to the cell membrane, which plays a role in segregation of daughter chromosomes at cell division, analogous to the role of the mitotic apparatus of eukaryotes. The membrane is the site of synthesis of DNA, cell wall polymers, and membrane lipids. It contains the entire electron transport system of the cell (and, hence, is functionally analogous to the mitochondria of eukaryotes). It contains receptor proteins that function in chemotaxis. Like cell membranes of eukaryotes, it is a permeability barrier and contains proteins involved in selective and active transport of solutes. It is also involved in secretion to the exterior of proteins (exoproteins), including exotoxins and hydrolytic enzymes involved in the pathogenesis of disease. The bacterial cell membrane is therefore the functional equivalent of most of the organelles of the eukaryotic cell and is vital to the growth and maintenance of the cell.

Basic structure of cell membrane is phospholipid–protein bilayer, usually lacking sterols

Membrane has roles in synthetic, homeostatic, secretory, and electron transport processes, and in cell division

Cell membrane is functional equivalent of many eukaryotic organelles

The cell membranes of Gram-positive and Gram-negative cells are similar in composition, structure, and function except for the modification, already described, in Gram-negative cells that places the outer membrane of the wall and the cell membrane in intimate contact (Bayer's junctions).

## Flagella

Flagella are molecular organelles of motility found in many species of bacteria, both Gram positive and Gram negative. They may be distributed around the cell (an arrangement called

Flagella are rotating helical protein structures responsible for locomotion

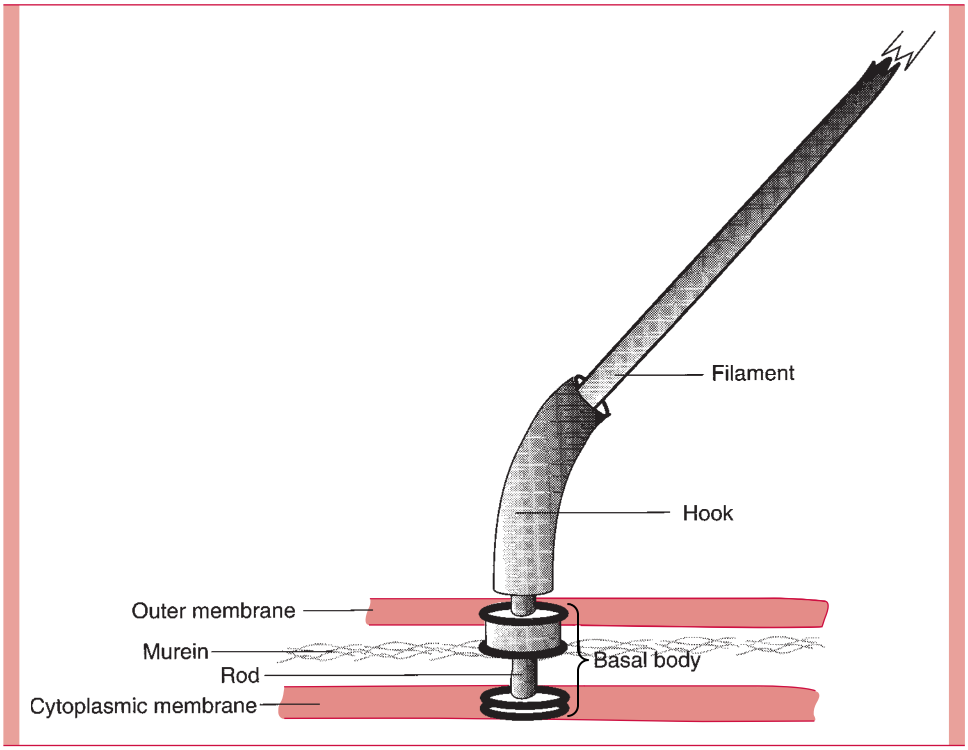


FIGURE 2–11
Schematic representation of the flagellar apparatus. (*After DePamphilis ML, Adler J. Fine structure and isolation of hook-basal body complex of flagella from* Escherichia coli *and* Bacillus subtilis. J Bacteriol *1971;105:384–359.*)

peritrichous from the Greek **trichos** for "hair"), at one pole (**polar** or **monotrichous**), or at both ends of the cell (**lophotrichous**). In all cases, they are individually helical in shape and propel the cell by rotating at the point of insertion in the cell envelope. The presence or absence of flagella and their position are important taxonomic characteristics.

Flagella have bushing rings in cell envelope

Flagellar filament is composed of the protein flagellin

The flagellar apparatus is complex, but consists entirely of proteins, encoded in genes called *fla* (for flagella). They are attached to the cell by a **basal body** consisting of several proteins organized as rings on a central rod (see Fig 2–5). In Gram-negative cells, there are four rings: an outer pair that serve as bushings through the outer membrane and an inner pair located in the peptidoglycan gel and the cell membrane. In Gram-positive cells, only the inner pair is present. The **hook** consists of other proteins organized as a bent structure that may function as a universal joint. Finally, the long **filament** consists of polymerized molecules of a single protein species called **flagellin** (Fig 2–11). Flagellin varies in amino acid sequence from strain to strain. This makes flagella useful surface antigens for strain differentiation, particularly among the Enterobacteriaceae.

Motility and chemotaxis, both important properties contributing to colonization, are discussed in Chapter 3.

## Pili

Pili are proteinaceous hair-like projections

Common pili have adherence roles

Male Gram-negative cells of some species have single tubular sex pili

Pili are molecular hair-like projections found on the surface of cells of many Gram-positive and Gram-negative species. They are composed of molecules of a protein called **pilin** arranged to form a tube with a minute, hollow core. There are two general classes, common pili and sex pili (Fig 2–12). **Common pili** cover the surface of the cell. They are, in many cases, **adhesins,** which are responsible for the ability of bacteria to colonize surfaces and cells. To cite only one example, the pili of *Neisseria gonorrhoeae* are necessary for the attachment to the urethral epithelial cells prior to penetration; without pili, the bacterium cannot cause gonorrhea. Thus, common pili are often important virulence factors. In fact, there are at least five different types of common pili (see Chapter 10). Some bacteriologists use the name **fimbriae** to refer to common pili. The sex pilus is diagnostic of a male bacterium and is involved in exchange of genetic material between some Gram-negative bacteria. There is only one per cell. The function of the sex pilus is discussed in Chapter 4.

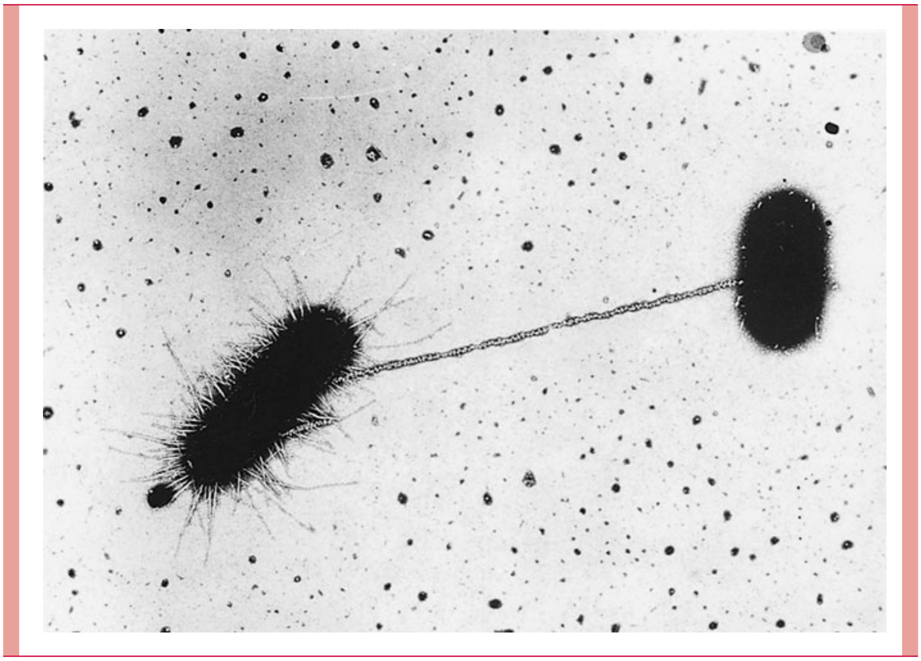

FIGURE 2–12
On the left-hand side is a "male" *Escherichia coli* cell exhibiting many common (somatic) pili and a sex pilus by which it has attached itself to a "female" cell that lacks the plasmid encoding the sex pilus. As discussed in Chapter 4, the sex pilus facilitates exchange of genetic material between the male and female *E. coli*. In this preparation, the sex pilus has been labeled with a bacterial virus that attaches to it specifically. (*Courtesy of Charles C. Brinton and Judith Carnahan.*)

## CORE

In contrast to the structural richness of the layers and appendages of the cell envelope, the interior seems relatively simple in transmission electron micrographs of thin sections of bacteria (Fig 2–13). There are two clearly visible regions, one granular (the cytosol) and one fibrous (the nucleoid). In addition, many bacteria possess plasmids that are usually circular, double-stranded DNA bodies in the cytosol separate from the larger nucleoid; plasmids are too small to be visible in thin sections of bacteria.

### Cytosol

Cytosol contains 70 S ribosomes and most of cell's metabolic enzymes

The dense **cytosol** is bounded by the cell membrane. It appears granular because it is densely packed with ribosomes, which are much more abundant than in the cytoplasm of eukaryotic cells. This is a reflection of the higher growth rate of bacteria. Each ribosome is a ribonucleoprotein particle consisting of three species of rRNA (5 S, 16 S, and 23 S) and about 56 proteins. The overall subunit structure (one 50 S plus one 30 S particle) of the 70 S bacterial ribosome resembles that of eukaryotic ribosomes (which are 80 S, composed of one 60 S and one 40 S particle), but is smaller and differs sufficiently in function that a very large number of antimicrobics have the prokaryotic ribosome as their target.

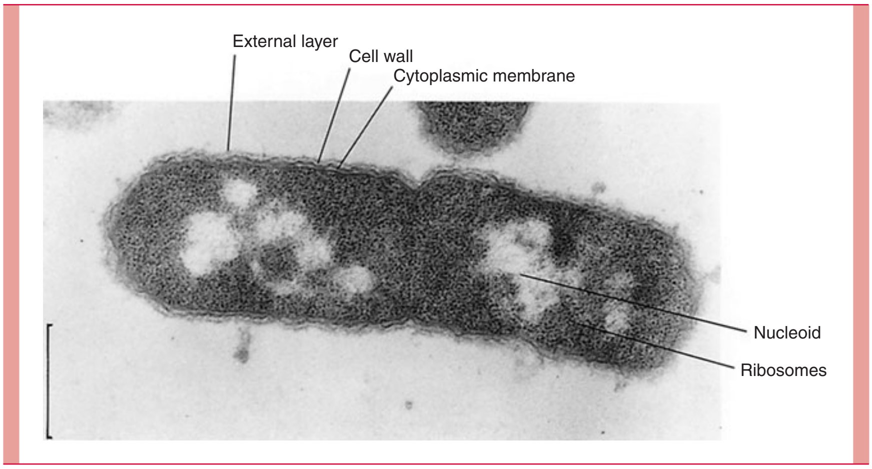


FIGURE 2–13
Electron micrograph of a Gram-negative bacterium. (*Courtesy of the late Dr. E. S. Boatman.*)

The number of ribosomes varies directly with the growth rate of the cell (see Chapter 3). At all but the slowest growth rates about 70% of the ribosomes at any one time exist as polysomes and are engaged in translating mRNA. Except for the functions associated with the cell membrane, all of the metabolic reactions of the cell take place in the cytosol. Accordingly, it is found to be the major location of a great fraction of the 2000 to 3000 different enzymes of the cell. The cytosol of some bacterial species also contains nutritional storage granules called **reserve granules.** The most prevalent kinds consist of glycogen or polymetaphosphate. Their presence and abundance depend on the nutritional state of the cell.

## Nucleoid

Nucleoid consists of a large tightly packed circular chromosome of supercoiled double-stranded DNA

Bacteria have no nuclear membrane

Nucleoid may be attached to cell membrane and central structures

The bacterial genome resides on a single chromosome (there are rare exceptions) and typically consists of about 4000 genes encoded in one, large, circular molecule of double-stranded DNA containing about 5 million nucleotide base pairs. This molecule is more than 1 mm long, and it therefore exceeds the length of the cell by some 1000 times. Needless to say, tight packing is necessary, and it is this packing that displaces all ribosomes and other cytosol components from the regions that appear clear or fibrous in electron micrographs of thin sections of bacterial cells (see Fig 2–13). Each region thus contains a chromosome, coated usually by polyamines and some specialized DNA-binding proteins but not with the structural organization of a eukaryotic chromosome. Because it is not surrounded by a membrane, it is not correctly called a nucleus but rather a **nucleoid** or **nuclear body.** The manner in which the DNA molecule is packed to form a nucleoid is not yet totally known. The double-helical DNA chain is twisted into supercoils, and it is suspected that the DNA is attached to the cell membrane. Evidence indicates that the entire chromosome is attached to some central structure, perhaps RNA, at a large number of points (12 to 80), creating folds of DNA, each of which is independently coiled into a tight bundle. Gentle methods of lysing cells permit nucleoids to be isolated as compact particles from which DNA loops can be sprung out.

Cell may contain 2–4 nucleoids depending on growth rate

Each nuclear body corresponds to a DNA molecule. The number of nuclear bodies varies as a function of growth rate; resting cells have only one, rapidly growing cells may have as many as four. As is described in Chapter 4, bacteria are genetically **haploid** for two reasons: (1) because all the chromosomes are identical and are segregated at random into daughter cells, and (2) because when rapidly growing cells slow down and form resting cells, the latter have returned to a single chromosome state.

The absence of a nuclear membrane confers on the prokaryotic cell a great advantage for rapid growth in changing environments. As described in Chapter 3, ribosomes can be translating mRNA molecules even as the latter are being made; no transport of mRNA from where it is made to where it functions is needed.

## Plasmids

Plasmids are small, usually circular, double-stranded DNA molecules

Plasmids may encode protective enzymes, virulence determinants, and self-transmissibility

Many bacteria contain small, usually circular, covalently closed, double-stranded DNA molecules separate from the chromosome. More than one type of plasmid or several copies of a single plasmid may be present in the cell. Many plasmids carry genes coding for the production of enzymes that protect the cell from toxic substances. For example, antibiotic resistance is often plasmid determined. Many attributes of virulence, such as production of some pili and of some exotoxins, are also determined by plasmid genes. Some plasmids code for production of a sex pilus by which they promote cell conjugation and thereby accomplish their own intercellular transmission. They are thus “infectious,” are nonhomologous to the bacterial chromosome, and provide a rapid method for acquisition of valuable genetic traits. This topic is considered in more detail in Chapter 4.

# SPORES

Endospores are hardy, quiescent forms of some Gram-positive bacteria, including important pathogens

**Endospores** are small, dehydrated, metabolically quiescent forms that are produced by some bacteria in response to nutrient limitation or a related sign that tough times are coming. Very few species produce spores (the term is loosely used as equivalent to endospores), but they are particularly prevalent in the environment. Some spore-forming bacteria are of great importance in medicine, causing such diseases as anthrax, gas gangrene, tetanus, and botulism. All spore formers are Gram-positive rods. Some grow only in the absence of oxygen (eg, *Clostridium tetani*), some only in its presence (eg, *Bacillus subtilis*).

Spore-forming allows survival under adverse conditions

The bacterial endospore is not a reproductive structure. One cell forms one spore under adverse conditions (the process is called **sporulation**). The spore may persist for a long time (centuries) and then, on appropriate stimulation, give rise to a single bacterial cell (**germination**). Spores, therefore, are survival rather than reproductive devices.

Endospore is not a reproductive structure

Spores of some species can withstand extremes of pH and temperature, including boiling water, for surprising periods of time. The thermal resistance is brought about by the low water content and the presence of a large amount of a substance found only in spores, **calcium dipicolinate.** Resistance to chemicals and, to some extent, radiation is aided by extremely tough, special coats surrounding the spore. These include a **spore membrane** (equivalent to the former cell membrane); a thick **cortex** composed of a special form of peptidoglycan; a **coat** consisting of a cysteine-rich, keratin-like, insoluble structural protein; and, finally, an external lipoprotein and carbohydrate layer called an **exosporium.**

Resistance of spore is due to dehydrated state, calcium dipicolinate, and specialized coats

Germination reproduces cell identical to that which sporulated

Sporulation is under active investigation. The molecular process by which a cell produces a highly differentiated product that is incapable of immediate growth but able to sustain growth after prolonged periods (centuries, in some cases) of nongrowth under extreme conditions of heat, desiccation, and starvation is of great interest. In general, the process involves the initial walling off of a nucleoid and its surrounding cytosol by invagination of the cell membrane, with later additions of special spore layers. Germination begins with activation by heat, acid, and reducing conditions. Initiation of germination eventually leads to outgrowth of a new vegetative cell of the same genotype as the cell that produced the spore.

## ADDITIONAL READING

Neidhardt FC, Ingraham JL, Schaechter M. *Physiology of the Bacterial Cell: A Molecular Approach.* Sunderland, MA: Sinauer Associates; 1990. A very readable description of the composition, organization, and structure of the bacterial cell is presented in Chapters 1 and 2. A good list of references for further reading is included.

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CHAPTER 3

# Bacterial Processes

FREDERICK C. NEIDHARDT

This chapter examines how the structural and chemical components of bacteria function in the growth and survival of these cells and in their colonization of the human host.

## CELL GROWTH

Growth of bacteria is accomplished by an orderly progress of metabolic processes followed by cell division by binary fission. Therefore, growth requires three complex processes: **metabolism,** which produces cell material from the nutrient substances present in the environment; **regulation,** which coordinates the progress of the hundreds of independent biochemical processes of metabolism to result in an orderly and efficient synthesis of cell components and structures in the right proportions; and **cell division,** which results in the formation of two independent living units from one.

Bacterial growth requires metabolism, regulation, and division by binary fission

### Bacterial Metabolism

We do not review in depth the many aspects of (mostly mammalian) metabolism customarily learned in biochemistry courses. Many of the principles, and even some of the details of metabolism, are universal. Indeed, the principle known as the **unity of biochemistry** is underscored by the fact that much of what we know of metabolic pathways is derived from work with *Escherichia coli.* We focus, rather, on the unique aspects of bacterial metabolism that are important in medicine.

The broad differences between bacteria and human eukaryotic cells can be summarized as follows:

1. The metabolism of most bacteria is geared to rapid growth and proceeds 10 to 100 times faster than in cells of our bodies.
2. Bacteria are much more versatile than human cells in their ability to use various compounds as energy sources and in their ability to use oxidants other than molecular oxygen in their metabolism of foodstuffs.
3. Bacteria are much more diverse than human cells in their nutritional requirements, because they are more diverse with respect to the completeness of their biosynthetic pathways.
4. The simpler prokaryotic body plan makes it possible for bacteria to synthesize macromolecules by far more streamlined means than our cells employ.
5. Some biosynthetic processes, such as those producing murein, lipopolysaccharide (LPS), and teichoic acid, are unique to bacteria.

Metabolism of prokaryotic cells is more active, versatile, and diverse than that of human cells

Each of these differences contributes to the special nature of the human–microbe encounter, and each provides a potential means for designing therapeutic agents to modify the outcome of this interaction.

Metabolic reactions accomplish four functions for growth: fueling, biosynthesis, polymerization, and assembly

Bacterial metabolism is highly complex. The bacterial cell synthesizes itself and generates energy for active transport, motility (in some species), and other activities by as many as 2000 chemical reactions. These reactions can be helpfully classified according to their function in the metabolic processes of **fueling, biosynthesis, polymerization,** and **assembly.**

## Fueling Reactions

Fueling reactions begin with the entry of substrates

**Fueling reactions** provide the cell with energy and with the 12 precursor metabolites used in biosynthetic reactions (Fig 3–1).

Nutrients enter despite envelopes that serve as permeability barriers

The first step is the capture of nutrients from the environment. Both Gram-positive and Gram-negative cells have surrounded themselves with envelopes designed in part to exclude potentially harmful substances and, therefore, have had to evolve a number of ways to ensure rapid transport of selected solute molecules through the envelope. Methods used by Gram-negative cells are summarized in Figure 3–2.

Facilitated diffusion involves shuttling by carrier protein

Almost no important nutrients enter the cell by **simple diffusion,** because the cell membrane is too effective a barrier to most molecules (the exceptions are carbon dioxide, oxygen, and water). Some transport occurs by **facilitated diffusion** in which a protein carrier in the cell membrane, specific for a given compound, participates in the shuttling of molecules of that substance from one side of the membrane to the other. Glycerol enters *E. coli* cells in this manner, and in bacteria that grow in the absence of oxygen (anaerobic bacteria, see below) it is reasonably common for some nutrients to enter the cell and for fermentation byproducts to leave the cell by facilitated diffusion. Because no energy is involved, this process can work only with, never against, a concentration gradient of the given solute.

Active transport can move nutrients against concentration gradient

Shock-sensitive transport involves periplasmic binding proteins and ATP-derived energy

**Active transport,** like facilitated diffusion, involves specific protein molecules as carriers of particular solutes, but the process is energy linked and can therefore establish a concentration gradient (active transport can pump "uphill"). Active transport is the most common mechanism in aerobic bacteria. Gram-negative bacteria have two kinds of active transport systems. In one, called **shock-sensitive** because the working components can be released from the cell by osmotic shock treatments, solute molecules cross the outer membrane either by diffusion through the pores of the outer membrane (as in the case of galactose) or by a special protein carrier (as in the case of maltose). In the periplasm, the solute molecules bind to specific **binding proteins,** which interact with carrier proteins in

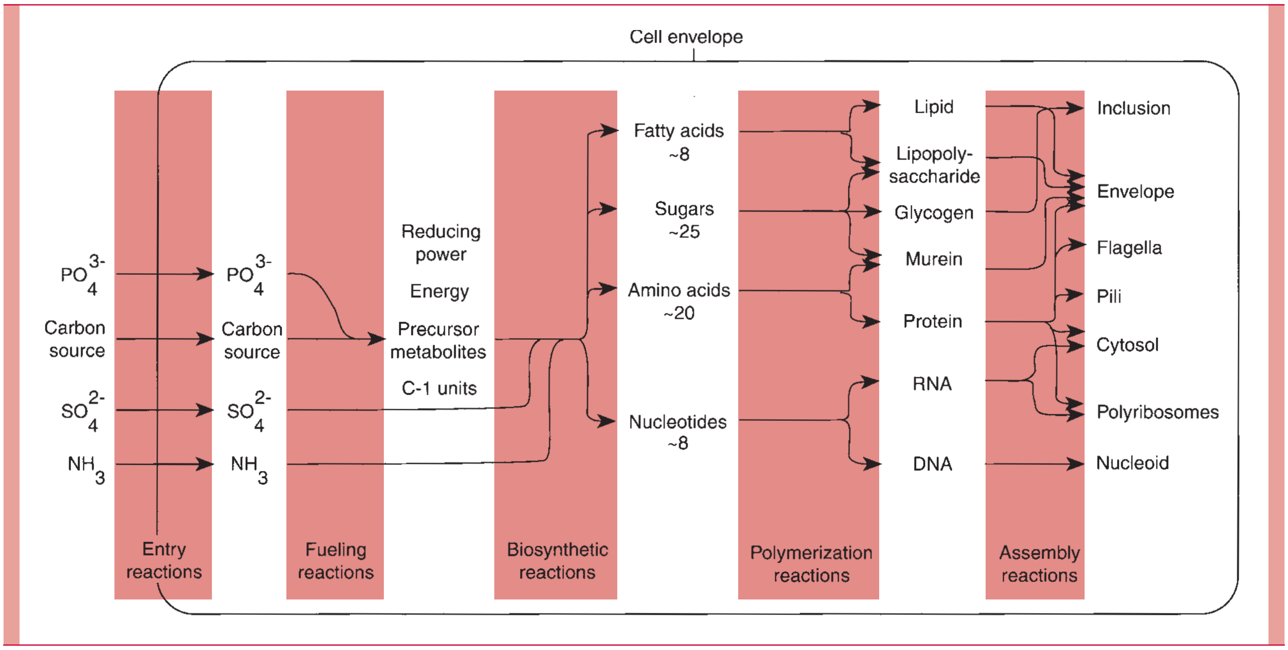


FIGURE 3–1
General pattern of metabolism leading to the synthesis of a bacterial cell from glucose.

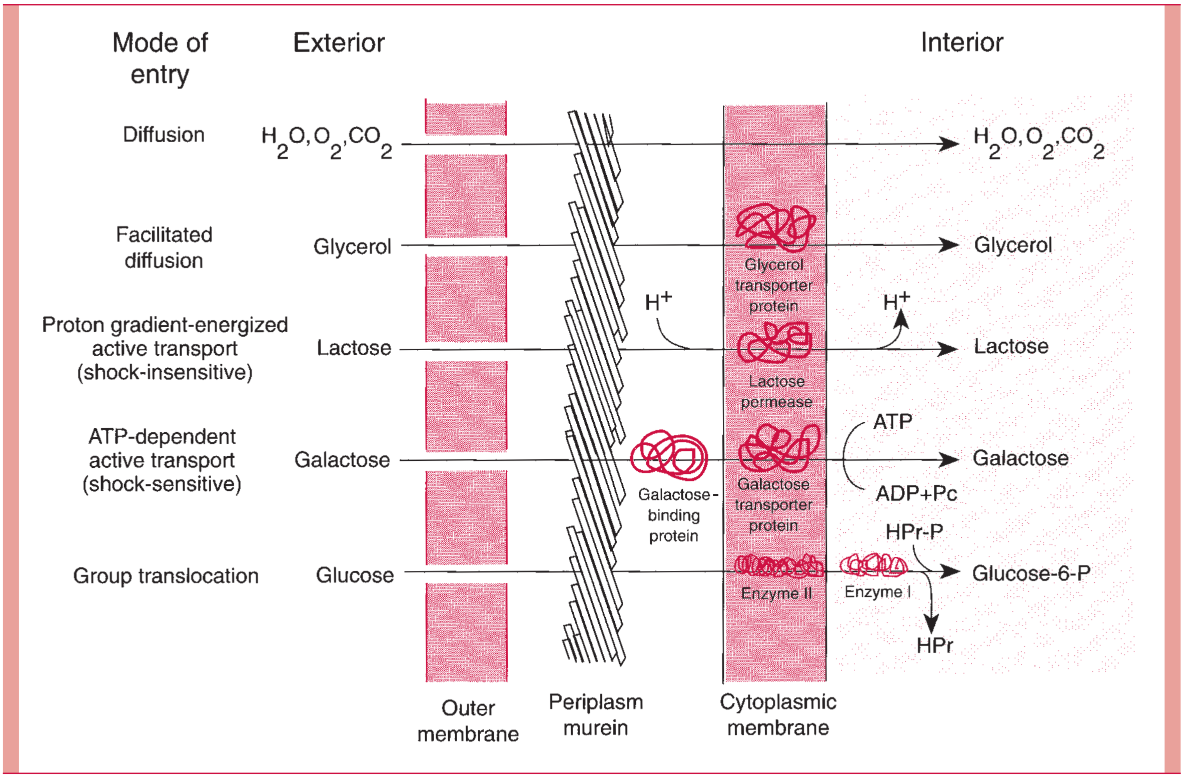


FIGURE 3–2
Schematic representation of the various modes of carbohydrate transport by *Escherichia coli.* Facilitated diffusion is demonstrated by glycerol transport; proton gradient-energized transport, by lactose uptake; shock-sensitive ATP-dependent transport, by galactose uptake; and group translocation, by glucose uptake.

the cell membrane. Shock-sensitive systems couple the transport across the cell membrane with the hydrolysis of ATP.

The other type of active transport involves only cell membrane components (and hence is **shock insensitive**) and is distinctive in that solute transport is coupled to the simultaneous passage of protons ($H^+$) through the membrane. The energy for this type of active transport is therefore derived not from ATP hydrolysis but from the proton gradient set up by electron transport within the energized cell membrane.

Shock-insensitive transport requires proton gradient energy

Finally, **group translocation** is an extremely common means of transport in the absence of oxygen. It involves the chemical conversion of the solute into another molecule as it is transported. The phosphotransferase system for sugar transport, which involves the phosphorylation of sugars such as glucose by specific enzymes, is a good example.

Group translocation involves chemical conversion of transported molecule

The transport of iron and other metal ions needed in small amounts for growth is special and of particular importance in virulence. There is little free $Fe^{3+}$ in human blood or other body fluids, because it is sequestered by iron-binding proteins (eg, **transferrin** in blood and **lactoferrin** in secretions). Bacteria must have iron to grow, and their colonization of the human host requires capture of iron. Bacteria secrete **siderophores** (iron-specific chelators) to trap $Fe^{3+}$; the iron-containing chelator is then transported into the bacterium by specific active transport. One example of a siderophore is **aerobactin** (a citrate type of hydoxamate), another is **enterobactin** (a catechol). Some siderophores are produced as a result of enzymes encoded not in the bacterial genome, but in the genome of a plasmid, providing another example of the many ways in which plasmids are involved in virulence.

Iron is an essential nutrient but is sequestered by host Fe-binding proteins

Bacterial siderophores chelate iron and are actively transported into cell

Once inside the cell, sugar molecules or other sources of carbon and energy are metabolized by the Embden–Meyerhof glycolytic pathway, the pentose phosphate pathway, and the Krebs cycle to yield the carbon compounds needed for biosynthesis.

Central fueling pathways produce biosynthetic precursors

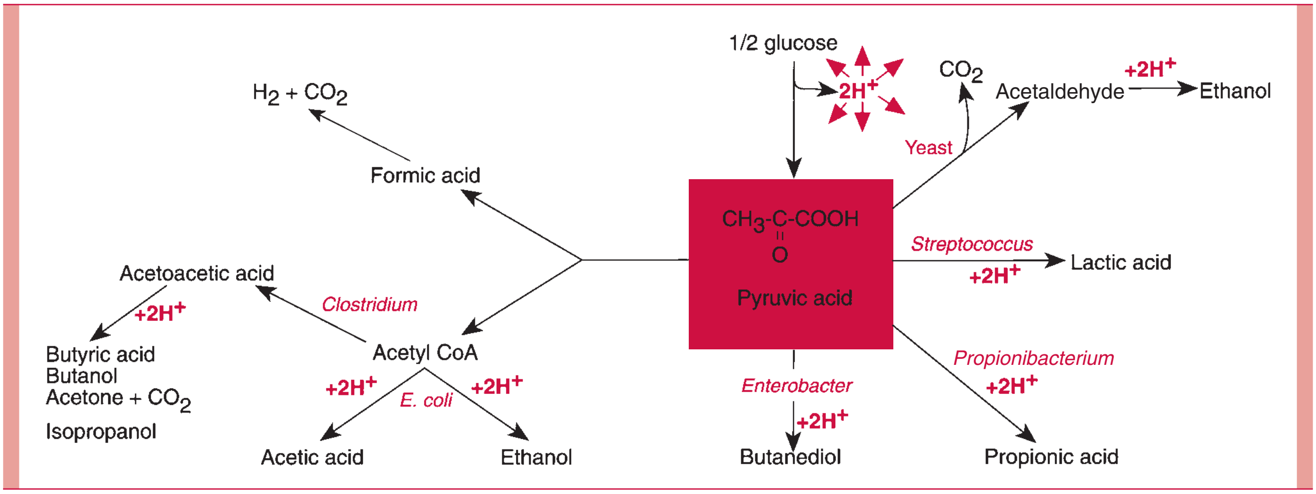


FIGURE 3–3
Some pathways of fermentation of sugars by various microorganisms. The protons ($H^+$) generated by the conversion of glucose to pyruvate by the Embden–Meyerhoff pathway are transferred to NAD. Oxidized NAD must be regenerated by reducing pyruvate and its derivatives.

Some bacteria have central fueling pathways (eg, the Entner–Doudoroff pathway) other than those familiar in mammalian metabolism.

Fermentation and respiration pathways each regenerate ATP and NAD$^+$

Working in concert, the central fueling pathways produce the 12 precursor metabolites. Connections to **fermentation** and **respiration** pathways allow the reoxidation of reduced coenzyme nicotinamide adenine dinucleotide (NADH) to NAD$^+$ and the generation of ATP. Bacteria make ATP by substrate phosphorylation in fermentation or by a combination of substrate phosphorylation and oxidative phosphorylation in respiration. (Photosynthetic bacteria are not important in medicine.)

Fermentation uses direct transfer of proton and electron to final organic receptor and produces organic acids and alcohols

It has low ATP-generating efficiency

**Fermentation** is the transfer of electrons and protons via NAD$^+$ directly to an organic acceptor. Pyruvate occupies a pivotal role in fermentation (Fig 3–3). Fermentation is an inefficient way to generate ATP, and consequently huge amounts of sugar must be fermented to satisfy the growth requirements of bacteria anaerobically. Large amounts of organic acids and alcohols are produced in fermentation. Which compounds are produced depends on the particular pathway of fermentation employed by a given species, and therefore the profile of fermentation products is a diagnostic aid in the clinical laboratory.

Respiration uses chain of electron carriers for which oxygen is usually but not always the terminal acceptor

**Respiration** involves fueling pathways in which substrate oxidation is coupled to the transport of electrons through a chain of carriers to some ultimate acceptor, which is frequently, but not always, molecular oxygen. Other inorganic (eg, nitrate) as well as organic compounds (eg, succinate) can serve as the final electron acceptor, and therefore many organisms that cannot ferment can live in the absence of oxygen (eg, *Pseudomonas aeruginosa* in the human colon).

Respiration is efficient energy producer

Respiration produces a proton-motive force that can generate ATP and power motility and active transport

Respiration is an efficient generator of ATP. Respiration in prokaryotes as in eukaryotes occurs by membrane-bound enzymes (quinones, cytochromes, and terminal oxidases), but in prokaryotes the cell membrane rather than mitochondrial membranes provide the physical site. The passage of electrons through the carriers is accompanied by the secretion from the cell of protons, generating an $H^+$ differential between the external surface of the cytosol membrane and the cell interior. This differential, called the **proton-motive force,** can then be used to (1) drive transport of solutes by the shock-insensitive systems of active transport (see above); (2) power the flagellar motors that rotate the filaments and result in cell motility in the case of motile species; and (3) generate ATP by coupling the phosphorylation of adenosine diphosphate (ADP) to the passage of protons inward through special channels in the cell membrane. The last pathway, facilitated by the enzyme anachronistically called **membrane ATPase,** can in fact function in either direction, coupling ADP phosphorylation to the inward passage of protons down the gradient or hydrolyzing ATP to accomplish the secretion of protons to establish a proton-motive force. The latter process explains how cells can generate a proton-motive force anaerobically (i.e., in the absence of electron transport).

In evolving to colonize every conceivable nook and cranny on this planet, bacteria have developed distinctive responses to oxygen. Bacteria are conveniently classified according to their fermentative and respiratory activities but much more generally by their overall response to the presence of oxygen. The response depends on their genetic ability to ferment or respire but also on their ability to protect themselves from the deleterious effects of oxygen.

Bacteria exhibit different characteristic responses to oxygen

Oxygen, though itself only mildly toxic, gives rise to at least two extremely reactive and toxic substances, **hydrogen peroxide** ($H_2O_2$) and the **superoxide anion** ($O^{2-}$). Peroxide is produced by reactions (catalyzed by flavoprotein oxidases) in which electrons and protons are transferred to $O_2$ as final acceptor. The superoxide radical is produced as an intermediate in most reactions that reduce molecular $O_2$. Superoxide is partially detoxified by an enzyme, **superoxide dismutase,** found in all organisms (prokaryotes and eukaryotes) that survive the presence of oxygen. Superoxide dismutase catalyzes the reaction

Aerobic metabolism produces peroxide and toxic oxygen radicals; aerobic growth is dependent on protective enzymes

Superoxide dismutase and peroxidase allow growth in air; their absence requires strict anaerobiosis

$$2O^{2-} + 2H^+ \rightarrow H_2O_2 + O_2.$$

Hydrogen peroxide is degraded by peroxidases by the reaction

$$H_2O_2 + H_2A \rightarrow 2H_2O + A$$

where A is any of a number of chemical groups (in the case in which $H_2A$ is another molecule of $H_2O_2$, the reaction yields $2H_2O + O_2$, and the peroxidase is called catalase). Bacteria that lack the ability to make superoxide dismutase and catalase are exquisitely sensitive to the presence of molecular oxygen and, in general, must grow anaerobically using fermentation. Bacteria that possess these protective enzymes can grow in the presence of oxygen, but whether they use the oxygen in metabolism or not depends on their ability to respire. Whether these oxygen-resistant bacteria can grow anaerobically depends on their ability to ferment.

Organisms growing in air may or may not have a respiratory pathway

Various combinations of these two characteristics (oxygen resistance and the ability to use molecular oxygen as a final acceptor) are represented in different species of bacteria, resulting in the five general classes shown in Table 3–1. There are important pathogens within each class. Both the nature of the diseases they cause and the methods for cultivating and identifying these pathogens in the laboratory are dictated to a large extent by their response to oxygen. Many medically important bacteria classified as anaerobes (including those listed in Table 3–1) are in fact moderately aerotolerant, and may possess low levels of superoxide dismutases and peroxidases that provide some survival protection, if not the ability to grow.

Important pathogens are found among aerobes, anaerobes, facultatives, indifferents, and microaerophiles

## Biosynthesis

Biosynthetic reactions form a network of pathways that lead from 12 precursor metabolites (provided by the fueling reactions) to the many amino acids, nucleotides, sugars, amino sugars, fatty acids, and other building blocks needed for macromolecules (see Fig 3–1). In addition to the carbon precursors, large quantities of reduced nicotinamide adenine dinucleotide phosphate (NADPH), ATP, amino nitrogen, and some source of sulfur are needed for biosynthesis of these building blocks. These pathways are similar in all species of living things, but bacterial species differ greatly as to which pathways they possess. Because all cells require the same building blocks, those that cannot be produced by a given cell must be obtained preformed from the environment. Nutritional requirements of bacteria, therefore, differ from species to species and serve as an important practical basis for laboratory identification.

Biosynthesis requires 12 precursor metabolites, energy, amino nitrogen, sulfur, and reducing power

Nutritional requirements differ depending on synthetic ability

Relatively few unique reactions in the domain of biosynthesis are present to form the basis for specific therapeutic attack on the microorganism rather than the host. The effectiveness of sulfonamides and trimethoprim is one of these exceptional situations; many

Only a few antimicrobics target biosynthetic processes

TABLE 3 – 1

## Classification of Bacteria by Response to Oxygen

| Type of Bacteria | Growth Response: Aerobic | Growth Response: Anaerobic | Possession of Catalase and Superoxide Dismutase | Comment | Example |
|---|---|---|---|---|---|
| Aerobe (strict aerobe) | + | − | + | Requires $O_2$; cannot ferment | *Mycobacterium tuberculosis*<br>*Pseudomonas aeruginosa*<br>*Bacillus subtilis* |
| Anaerobe (strict anaerobe) | − | + | − | Killed by $O_2$; ferments in absence of $O_2$ | *Clostridium botulinum*<br>*Bacteroides melaninogenicus* |
| Facultative | + | + | + | Respires with $O_2$; ferments in absence of $O_2$ | *Escherichia coli*<br>*Shigella dysenteriae*<br>*Staphylococcus aureus* |
| Indifferent (aerotolerant anaerobe) | + | + | + | Ferments in presence or absence of $O_2$ | *Streptococcus pneumoniae*<br>*Streptococcus pyogenes* |
| Microaerophilic | (+)[a] | + | (+)[a] | Grows best at low $O_2$ concentration; can grow without $O_2$ | *Campylobacter jejuni* |

[a](+) indicates small amounts of growth or catalase and superoxide dismutase.

bacteria must synthesize folic acid rather than use it preformed from their environment, as human cells do, which renders these bacteria susceptible to agents that interfere with the biosynthesis of folic acid.

## Polymerization Reactions

Unlike fueling and biosynthetic processes, polymerization reactions offer many targets for antimicrobic chemotherapy. The reason is simple: the bacterial machineries for replication, transcription, and translation differ from that in the human host cells.

**Polymerization** of DNA is called **replication.** From studies largely made in *E. coli,* DNA replication involves 12 or more proteins acting at a small number of sites (replication forks) where DNA is synthesized from activated building blocks (dATP, dGTP, dCTP, and TTP). Replication always begins at special sites on the chromosome called *oriC* in *E. coli* (for origin of replication) and then proceeds bidirectionally around the circular chromosome (Fig 3–4). Synthesis of DNA at each replication fork is termed **semiconservative** because each of the DNA chains serves as the template for the synthesis of its complement, and, therefore, one of the two chains of the new double-stranded molecule is conserved from the original chromosome. One of the two new strands must be synthesized in chemically the opposite direction of the other; this is accomplished by having each new strand made in short segments, 5′ to 3′, which are then ligated by one of the DNA-synthesizing enzymes (see Fig 3–4). Interestingly, an RNA primer is involved in getting each of these segments initiated. The two replication forks meet at the opposite side of the circle. The frequency of initiation of chromosome replication (and, therefore, the number of growing points) varies with cell growth rate; the chain elongation rate is rather constant at a given temperature independent of cell growth rate.

Bidirectional, semiconservative replication occurs at replication forks, involves RNA primers, and proceeds at a pace largely independent of growth rate

Some chemotherapeutic agents derive their selective toxicity for bacteria from the unique features of prokaryotic DNA replication. The synthetic quinolone compounds inhibit DNA gyrase, one of the many enzymes participating in DNA replication.

DNA gyrase inhibitors are selectively toxic for bacteria

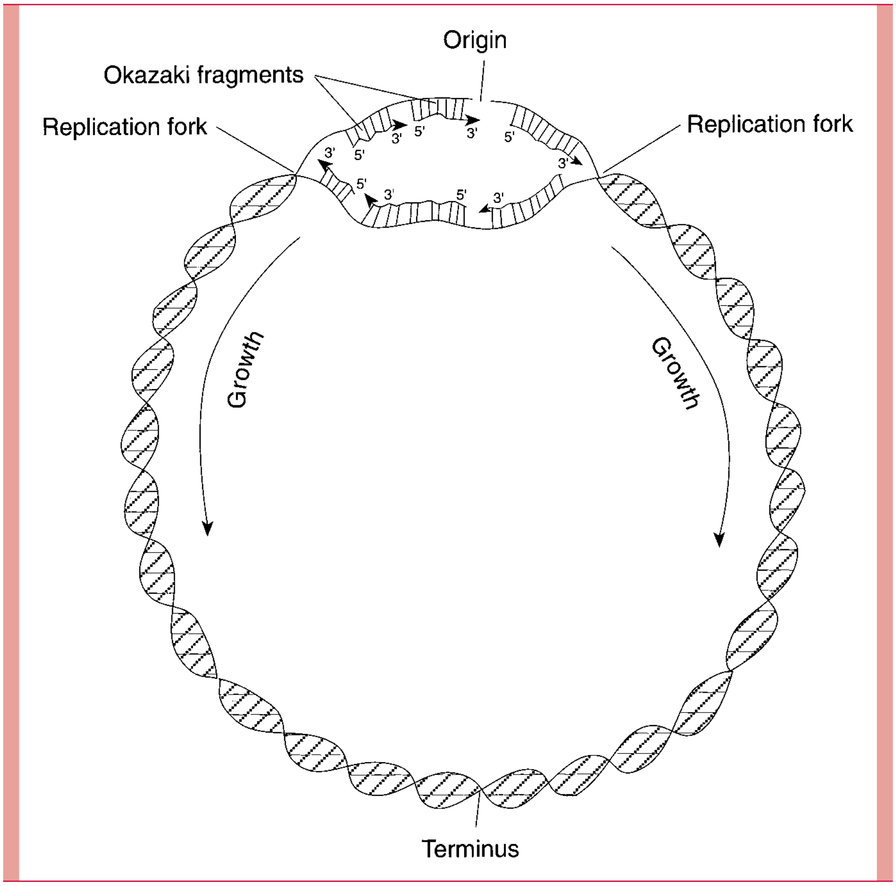


FIGURE 3–4
Schematic representation of DNA replication in bacteria. Shown is a portion of a replicating chromosome shortly after replication has begun at the origin. The newly polymerized strands of DNA are synthesized in the 5′ to 3′ direction (indicated by the arrows) using preexisting DNA strands as templates. The process creates two replication forks that travel in opposite directions until they meet on the opposite side of the chromosome.

A single RNA polymerase makes all forms of bacterial RNA

**Transcription** is the synthesis of RNA. Transcription in bacteria differs from that in eukaryotic cells in several ways. One difference is that all forms of bacterial RNA (mRNA, tRNA, and rRNA) are synthesized by the same enzyme, RNA polymerase. Like the several eukaryotic enzymes, the single bacterial RNA polymerase uses activated building blocks (ATP, GTP, CTP, and UTP) and synthesizes an RNA strand complementary to whichever strand of DNA is serving as template.

Bacterial mRNA needs no special transport to ribosomes

A second major difference is that bacterial mRNA need not be transported to the cytoplasm through a nuclear membrane, and hence no poly(A) cap is needed and no special means of transport exists. In fact, because each mRNA strand is directly accessible to ribosomes, binding of the latter to mRNA to form polysomes begins at an early stage in the synthesis of each mRNA molecule (Fig 3–5).

Bacteria constantly turn over their complement of mRNA

A third remarkable difference is that bacterial mRNA is synthesized, used, and degraded all in a matter of a few minutes. Although most bacteria have some long-lived species of mRNA, it is characteristic of bacterial cells to "wipe their [transcript] plate clean" every few minutes and make whatever new transcripts are called for by sensing the cell's environment.

$\sigma$ Subunit recognizes promoters

RNA polymerase is a large, complicated molecule with a subunit structure of $\alpha 2\beta\beta'\sigma$. The $\sigma$ subunit is the one that locates specific DNA sequences, called promoters, which precede all transcriptional units. More than one $\sigma$ subunit, each designed to recognize a different set of related promoters, can associate with RNA polymerase, which provides a simple means to activate groups of related genes that cooperate in such cellular

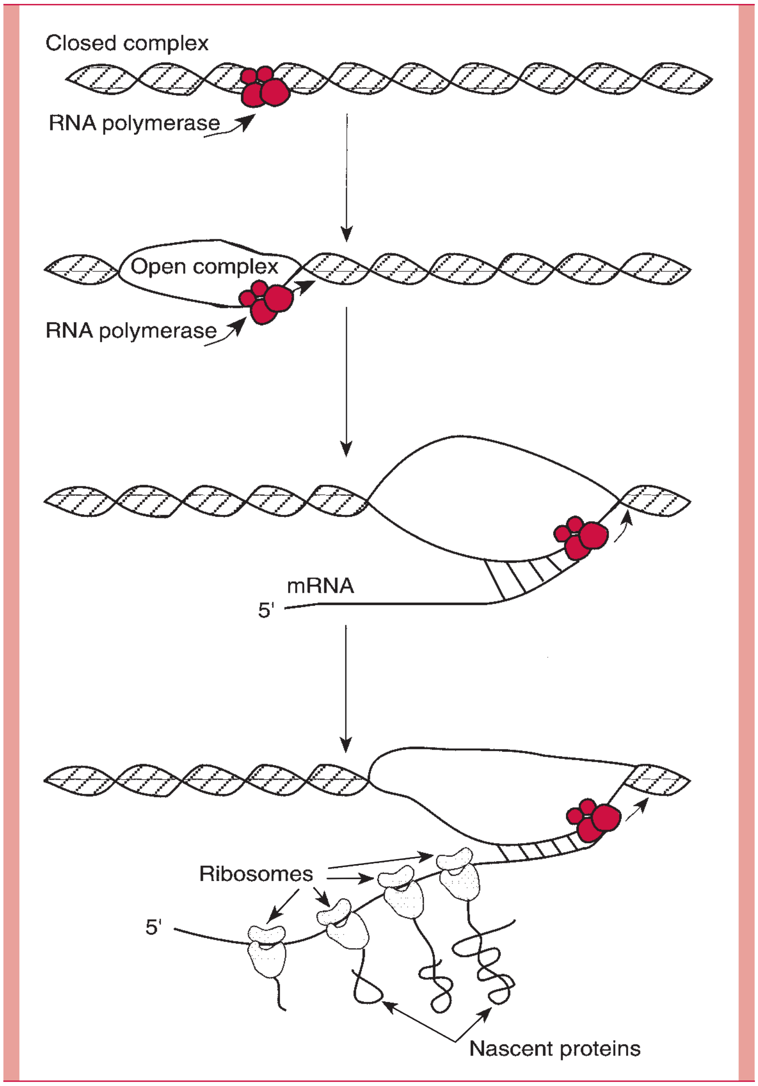


FIGURE 3–5
Schematic representation of the coupling of transcription and translation in bacteria.

processes as sporulation, nitrogen acquisition, heat shock stress response, and adaptation to nongrowth conditions.

As in eukaryotic cells, all stable RNA molecules are made from large precursor molecules that must be processed by nucleases and then extensively modified to produce the mature product (the tRNAs and rRNAs).

Bacterial RNA polymerase is the target of the **rifamycin** series of antimicrobics (including the semisynthetic compound **rifampin**). They block initiation of transcription. Other substances of biological origin block extension of RNA chains or inhibit transcription by binding to DNA. They have been of great value in molecular biological studies but are also toxic to human cells and thus are not used in human therapy.

Rifampin inhibits RNA polymerase

**Translation** is the name given to protein synthesis. Many antimicrobics derive their selective toxicity for bacteria from the unique features of the prokaryotic translation apparatus. In fact, protein synthesis is the target of a greater variety of antimicrobics than is any other metabolic process (see Chapter 13). Some agents inhibit the ribosomal large subunit (eg, **chloramphenicol** and **macrolides**), some the small subunit (eg, **tetracyclines** and **aminoglycosides**), and some aminoglycosides bind to both large and small subunits.

Many antibiotics act on bacterial translation machinery

Bacteria activate the 20-amino-acid building blocks of protein in the course of attaching them to specific transfer RNA molecules. The aminoacyl-tRNAs are brought to the ribosomes by soluble protein factors, and there the amino acids are polymerized into polypeptide chains according to the sequence of codons in the particular mRNA that is being translated. Having donated its amino acid, the tRNA is released from the ribosome to return for another aminoacylation cycle.

Amino acid residues are polymerized from specific tRNAs at the direction of mRNA

This description fits translation in eukaryotic as well as prokaryotic cells, but major differences do exist. The initiation of translation of a new polypeptide chain requires fewer proteins in bacteria. The ribosomes of bacteria are smaller and simpler in structure. Bacterial mRNA is largely polycistronic, that is, each mRNA molecule is the transcript of more than one gene (cistron) and therefore directs the synthesis of more than one polypeptide. No processing or transport of the mRNA is necessary. RNA polymerase makes mRNA at about 55 nucleotides per second (at 37°C), and ribosomes make polypeptide chains at about 18 amino acids per second. Therefore, not only does translation of each mRNA molecule occur simultaneously with transcription, but it occurs at the same linear rate (55 nucleotides per second/3 nucleotides per codon = 18 amino acids per second). This means that ribosomes are traveling along each mRNA molecule as fast as RNA polymerase makes it. This coupling plays a role in several aspects of regulation of gene expression unique to bacteria.

mRNA is polycistronic and requires no processing or transport

Translation of mRNA occurs simultaneously with transcription

These special features of translation in bacteria contribute to the streamlined efficiency of the process. The bacterial cytosol is packed with polyribosomes. Each ribosome functions near its maximal rate. Therefore, the faster the growth rate of the cell, the more ribosomes are needed for protein production. It can be estimated that during growth in rich media, more than half the mass of the *E. coli* cell consists of ribosomes and other parts of the translation machinery.

Bacteria synthesize proteins rapidly and efficiently

Other polymerization reactions involve synthesis of peptidoglycan, phospholipid, LPS, and capsular polysaccharide. All of these reactions involve activated building blocks that are polymerized or assembled within or on the exterior surface of the cytoplasmic membrane.

The entire process of synthesizing **peptidoglycan (murein),** which is completely absent from eukaryotic cells, offers many vulnerable attack points for antibiotics and other chemotherapeutic agents. Some of these are shown in Figure 3–6; others are described more fully in Chapter 13.

Uniqueness of wall offers many targets for antimicrobics

The synthesis of murein occurs in three compartments of the cell (see Fig 3–6).

1. In the cytosol a series of reactions leads to the synthesis, on a nucleotide carrier (UDP), of an *N*-acetylmuramic acid (NAM) residue bearing a pentapeptide (the tetrapeptide found in mature murein plus an additional terminal D-alanine).

NAM and attached peptide are synthesized in cytosol

2. This precursor is then attached, with the release of UMP, to a special, lipid-like carrier in the cell membrane called **bactoprenol** (or **undecaprenol**). Within the cell membrane *N*-acetylglucosamine (NAG) is added to the precursor, along with any amino

Precursor is added to bactoprenol carrier, and NAG and bridge amino acids are added in membrane

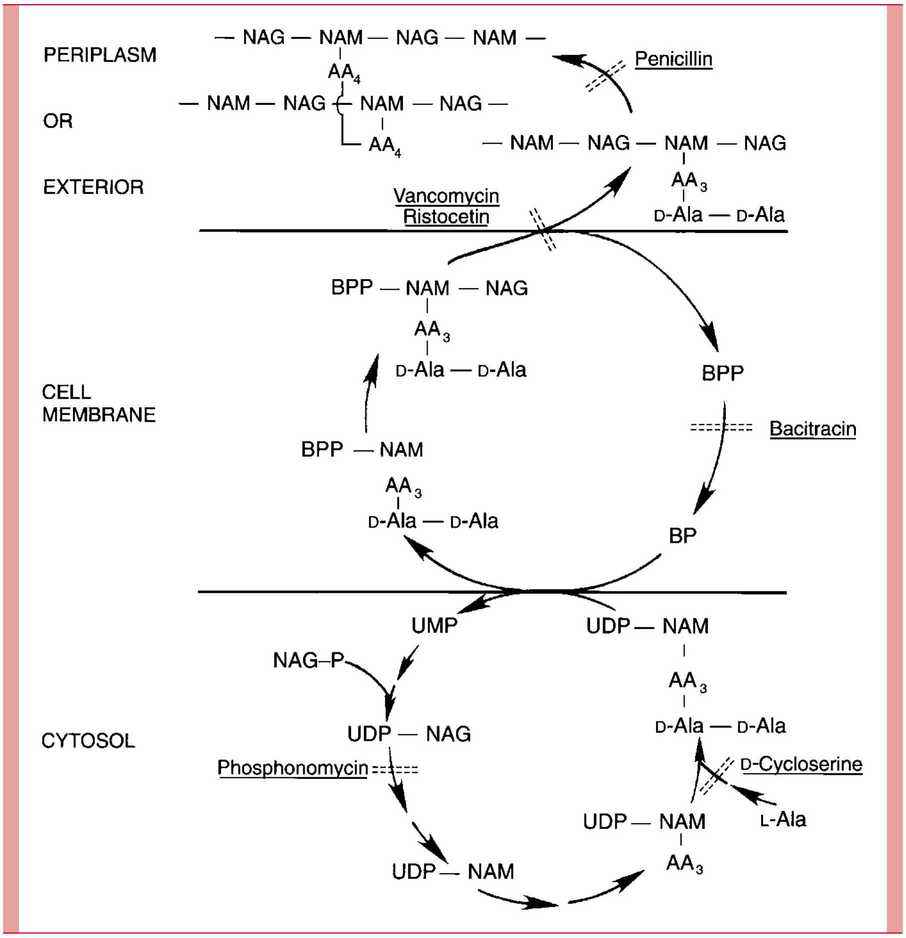


FIGURE 3–6
Schematic representation of murein synthesis with sites of action of some antibiotics. NAG, *N*-acetylglucosamine; NAM, *N*-acetylmuramic acid; BP and BPP, bactoprenol phosphate and bactoprenol pyrophosphate, respectively; $AA_3$, tripeptide residue that in *Escherichia coli* is L-alanyl-D-glutamyl-*m*-diaminopimelic acid; D-Ala and L-Ala, D-alanine and L-alanine, respectively; UMP and UDP, uridine mono- and diphosphate, respectively. Some of the arrows represent more than one chemical reaction. See the text for a description of this process.

acids that in this particular species will form the bridge between adjacent tetrapeptides. **Bacitracin** and **vancomycin** interfere with the function of bactoprenol as a carrier in polymerization and assembly reactions.

Glycan polymer and peptide cross-links are formed in periplasm or wall

PBPs are involved in assembly, expansion, and shaping of murein

3. Outside the cell membrane (in the periplasm of Gram-negative cells and the wall of Gram-positive cells), this disaccharide subunit is attached to the end of a growing glycan chain, and then cross-links between chains are formed by a transpeptidization using the energy transduced by the release of the terminal D-alanine—the extra amino acid on the tetrapeptide. Eventually, release from the carrier occurs. These transpeptidases, called **penicillin-binding proteins** (PBPs) for their property of combining with this antibiotic, are involved in forging, breaking, and reforging the peptide cross-links between glycan chains. This dynamic process is necessary to permit expansion of the murein sac during cellular growth, to shape the envelope, and to prepare for cell division. It is this process that goes awry in the presence of penicillin and related antimicrobics, the action of which can be broadly stated as preventing formation of stabilizing peptide cross-links.

## Assembly Reactions and Protein Translocation

Guided assembly involves transport of components within cell

Self-assembly (eg, of ribosomes) can be mimicked in vitro

Assembly of cell structures occurs both by spontaneous aggregation (**self-assembly**) and by special, specific mechanisms (**guided assembly**). Some macromolecules are made at the sites of assembly (such as LPS in the outer membrane), and others must be transported to them (porin is made in the cytosol but ends up in the outer membrane). Self-assembly is illustrated by two cell structures that spontaneously assemble in a test tube from their component macromolecules: flagella and ribosomes. Important parts of envelope assembly include special mechanisms for the secretion of proteins, the use of

Bayer's zones of adhesion (see Chapter 2, Fig 2–9) to form the phospholipid/protein leaflets of the membranes, and the use of carrier molecules (eg, bactoprenol) to transport hydrophilic compounds within the lipid portions of the membrane.

## *Translocation of Proteins*

A problem is posed by the difficulty of moving macromolecules out of the cell interior and into their proper place in the wall, outer membrane, and capsule. Proteins in their natural folded state present a hydrophilic surface that cannot be pushed through phospholipid membranes. These proteins may be part of the cell's assembly process, and are destined to reside within the membrane or wall of the cell, or in the case of Gram-negative cells to reside finally in either the periplasm or outer membrane. Moreover, many proteins are translocated through all layers of the cell envelope to the exterior environment. **Protein secretion** has become the general term to designate all these instances of translocation of proteins out of the cytosol (ie, whether the protein is to leave the cell or become part of the envelope), recognizing that all these events share the problem of passing a protein between hydrophilic and hydrophobic phases. An understanding of this complex process is beginning, and it turns out to have great relevance to bacterial virulence. Approximately 20% of the proteins of *E. coli* are estimated to reside in the envelope. Furthermore, many bacterial virulence factors are located on the surface of the cell, poised to interact with the cells and fluids of the mammalian host. Studies with *E. coli* and many other Gram-negative as well as Gram-positive bacteria have revealed a surprising number of mechanisms for protein translocation.

Proteins do not readily pass through membranes

Special mechanisms exist for protein translocation

Proteins destined for the wall, membranes, or periplasm are translocated by a **general secretory pathway (GSP),** which consists of cytosolic chaperones and an integral membrane **translocase** consisting of several proteins operating cooperatively. The role of the chaperones is to present the protein to be exported to the translocase, at which a special ATPase **"pusher"** is thought to physically drive the proteins through the membrane. Proteins of the GSP are products of what are called *sec* (secretory) genes, and the GSP is therefore also called the **Sec** pathway. Many, but not all, exported proteins are recognized by having a special **signal sequence** at their N-terminus; this peptide is cleaved off during translocation through the membrane by a **signal peptidase.** The translocation of some proteins occurs cotranslationally (ie, during their synthesis on a ribosome) before the polypeptide has a chance to fold. For some of these, the nascent polypeptide–ribosome complex is docked to the membrane by a **signal recognition particle,** similar to that in mammalian cells, consisting of a protein (Ffh) and a 4.5S RNA. For others, translocation occurs posttranslationally; the protein is completed and then may be escorted to the translocase by chaperone proteins. Some of these general aspects of protein translocation are shown in Figure 3–7.

The GSP or Sec system handles most protein translocation for cell assembly

Many components are products of *sec* genes

Chaperones, translocase proteins, and signal peptidase form the GSP

Many proteins are marked for translocation by being made with a signal sequence

## *Export of Proteins*

In many cases, proteins are translocated completely through the entire envelope and into the surrounding media or tissue, or even directly into host cells. Secretion of toxins and other proteins contributes greatly to bacterial virulence, and occurs by several pathways, only some of which utilize components of the GSP. In Gram-negative species, secretion must translocate a protein across two membranes. In Gram-positive species, secretion is less complex and usually involves proteins marked by a signal sequence interacting with chaperones and translocases with general similarity to those of the GSP. Five pathways have been discovered in different Gram-negative pathogens that accomplish export of proteins into the environment (Fig 3–8). These pathways are important because many of the secreted proteins are toxins or other virulence factors.

Secretion is more complex in Gram-negative than Gram-positive cells

Type I secretion systems are Sec-independent (do not use the GSP), and consist of three proteins that form a transmembrane channel through which the secreted protein moves, driven by one of the proteins, an ATP-binding cassette (**ABC**) **transporter;** hence, these systems are sometimes called simply **ABC transporters.** In a single step,

Type I secretion is by relatively simple ABC transporter systems independent of the Sec system

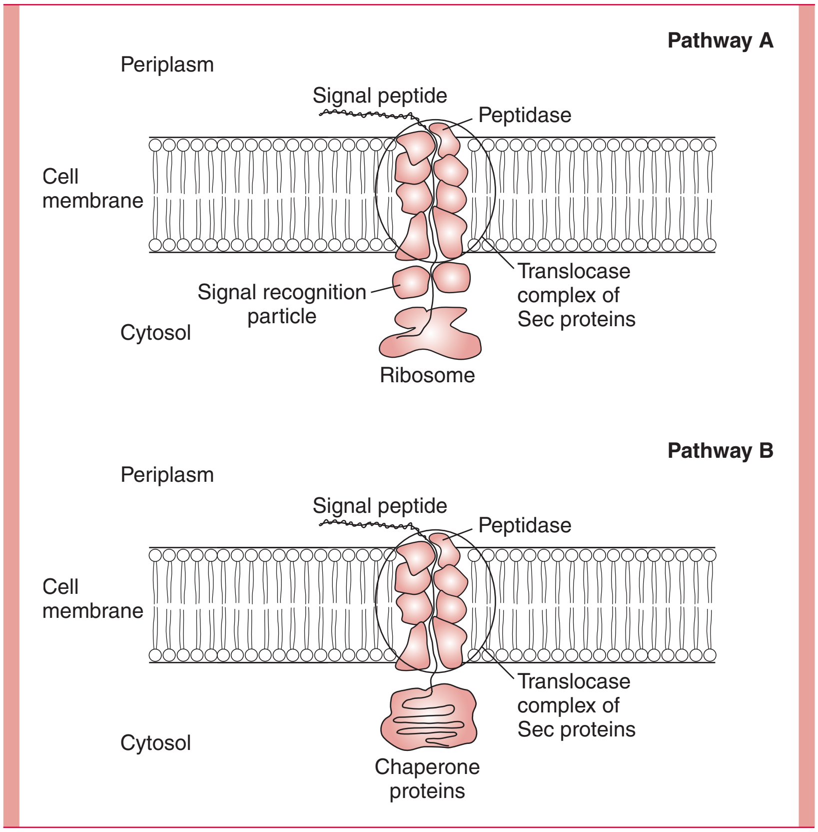


FIGURE 3–7
Translocation of proteins using the general secretory pathway (GSP). Pathway A depicts a protein being cotranslationally translocated, with the ribosome/polypeptide complex docked to the membrane by a signal recognition particle. Pathway B depicts a protein being posttranslationally translocated after protection in the cytosol by chaperone proteins.

the secreted protein, which normally lacks a classical signal sequence, passes from the cytosol to the external environment. The *E. coli* hemolysin is secreted in this manner.

Type II secretion is called two-step secretion; the first step occurs by GSP

Type II secretion systems, on the other hand, are Sec-dependent and use the traditional GSP to move a protein into the periplasm, but then in a second step, approximately 14 accessory protein molecules move the secreted protein across the outer membrane. This process is called **two-step secretion**. Like the type I systems, type II systems include an ATP-binding protein but also a peptidase to cleave a signal sequence from the secreted proteins, all of which have a signal sequence. These systems are common in such Gram-negative bacteria as *Klebsiella oxytoca, Vibrio cholerae, Pseudomonas aeruginosa,* and *E. coli*.

Type III secretion is called contact-dependent secretion and is Sec-independent

Type III secretion injects virulence proteins directly into human cells on contact

Type III systems, which are responsible for the secretion of many virulence factors in *Yersinia, Salmonella, Shigella,* and *Pseudomonas* species, involve as many as 20 protein components. One component is a chaperone specific for the given protein to be secreted, and another is an ATP-binding protein thought to energize the system. Type III systems are attracting intense study because they are responsible for **contact-dependent secretion,** in which secretion of virulence proteins is activated by contact with mammalian host cells, resulting in the direct injection of the secreted protein into the cytoplasm of the mammalian cell. Type III systems are Sec-independent.

Type IV secretion adapts a DNA-transfer system to proteins

Type IV systems are referred to as **conjugal transfer systems** because they were originally discovered as pathways by which DNA is conjugally transferred between bacterial cells or between a bacterial and a eukaryotic cell. They are used by the plant pathogen *Agrobacterium tumefaciens* to transfer oncogenic DNA and protein into plants, and a similar system is used by *Bordetella pertussis* to export pertussis (whooping cough) toxin. Genes similar to those responsible for this type of secretion in these organisms are found in the pathogenicity island of *Helicobacter pylori* and in *Legionella pneumophila*. Currently it is unclear whether the protein secretion by these systems requires the Sec machinery.

Type V secretion uses GSP for the first step; protein to be secreted accomplishes the second step

Type V secretion systems are two-step, Sec-dependent pathways. No helper protein is needed for translocation through the outer membrane; the transported protein itself accomplishes this feat. Hence, these systems are referred to as **autotransporters.** One

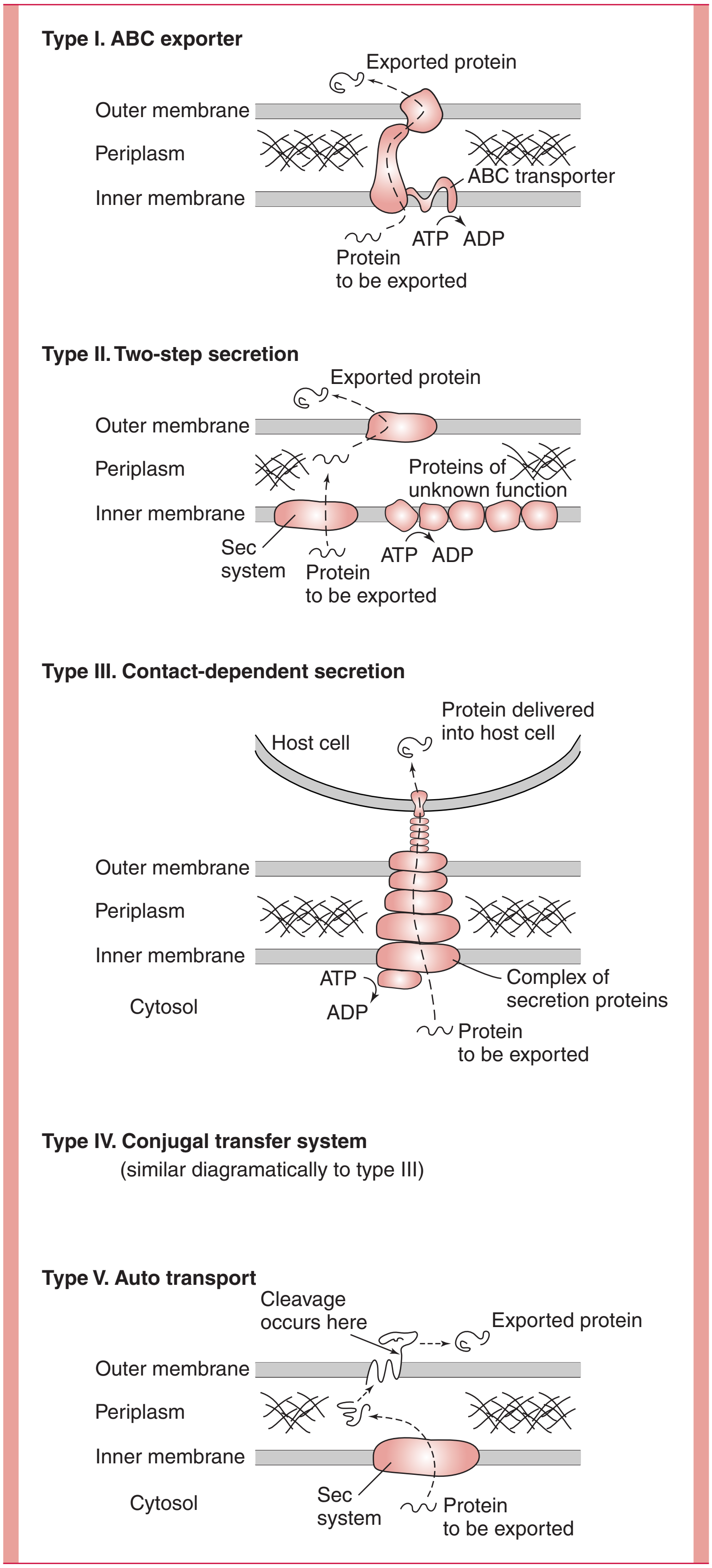


FIGURE 3–8
Simplified schematic diagrams that compare the main features of protein export by the five known pathways. (*Adapted from Harper JR, Silhavy TJ. Germ warfare: The mechanisms of virulence factor delivery. In: EA Groisman,* Principles of Bacterial Pathogenesis, *San Diego, CA: Academic Press; 2001, pp 43–74.*)

domain of the protein forms a channel in the outer membrane for the rest of the protein and then is cleaved off. These are the simplest of the Sec-dependent systems. A serine protease from *Serratia marcescens,* the important IgA proteases of *Haemophilus influenzae* and *Neisseria gonorrhoeae,* and the vacuolating cytotoxin VacA from *H. pylori* are each secreted as autotransporters.

Bacterial secretion and export offer potential targets for future design of chemotherapeutic agents.

## Cell Division

Many genes are involved in cell division

Multiple replication forks allow faster cell division than chromosome replication

Bacteria multiply by binary fission. More than 30 genes in *E. coli* are known to be involved in the process that involves the polar separation of the daughter chromosomes, the formation of the cross-wall and envelope at the point of cell division, and ultimately the separation of the two newly formed cells. In rich medium at 37°C, the entire process is completed in 20 minutes in *E. coli* and many other pathogenic species. The most astounding aspect of this feat is that the replication of the chromosome in these cells takes approximately 40 minutes, largely independently of the nature of the medium. The trick of dividing faster than the chromosome can replicate is accomplished by a mechanism that triggers the start of a new round of replication before an earlier one has been completed. In other words, during rapid growth multiple pairs of replication forks are at work on a given chromosome, and a newborn cell inherits chromosomes that have already been partially replicated. Bacteria maintain a constant cell mass:DNA ratio, and because rapidly growing cells have extra DNA (due to the multiple replication forks), cell size obviously is related to growth rate; the faster bacteria grow, the larger is their average size.

Anucleate cells are produced unless division and replication are coordinated

Cell division must be precisely coordinated with the completion of a round of DNA replication, or nonviable offspring will be produced. This coordination does not just happen; it requires a special regulatory system. Mutants are known that are defective in this regulation; in some of them, cell division without chromosome replication and segregation leads to the formation of **minicells,** which are complete cells save for lacking DNA.

Division and morphology are distorted by many antimicrobics

The complexity of cell division would lead one to expect that it might be easily disrupted by chemotherapeutic agents, and this is the case. Nonlethal concentrations of antimicrobics that act, even indirectly, on the polymerization or assembly reactions of the cell wall cause the formation of bizarre and distorted cells. Long filaments can result from incomplete cell division in the case of rod-shaped bacteria such as *E. coli.* Such forms are frequently encountered in direct examination of specimens from patients treated with antimicrobics.

# GROWTH OF BACTERIAL CULTURES

Pure cultures are produced by inoculating media with genetically identical cells

Growth on agar media yields visible colonies, each of which is a clone of cells if derived from a single cell

Colony size, form, and consistency are distinguishing features

Solutions of nutrients that support the growth of bacteria are called **media** (singular, **medium**), which can be solidified by the incorporation of agar. The introduction of live cells into liquid sterile media or onto the surface of solidified media is called **inoculation.** A population of bacterial cells is referred to as a **culture.** If the population is genetically homogeneous (ie, if all cells belong to the same strain of the same species), it is called a **pure culture.** Study of bacteria usually requires pure cultures, which can be obtained in several ways. The most common is to spread a very dilute suspension of a mixed culture on the surface of medium solidified with agar. Growth of individual cells deposited across the surface of solidified medium leads to visible mounds of bacterial mass called **colonies.** The cells in a colony are usually descended from a single original cell and, in this case, constitute a **clone.** There is little difference between a pure culture and a clone, except that a pure culture may have been produced by the original inoculation of several identical cells. Colonies of different species and strains show marked differences in size, form, and consistency resulting from differences in growth rates, surface properties of the organisms, and their response to the gradients of nutrients and metabolites that develop within the colony as it enlarges. This facilitates subculturing to pure cultures. The diagnostic application of these techniques is discussed in Chapter 15.

Growth of a liquid culture can be monitored by colony counts or turbidimetrically

Growth of a liquid bacterial culture can be monitored by removing samples at timed intervals and placing suitable dilutions in or on solidified medium to obtain a count of the number of colonies that develop. The count can be directly extrapolated to the number of viable units in the original sample (which, because certain bacteria clump or form chains, may not represent the number of bacterial cells). Growth can also be measured by determining the number of **total cells** in each sample. Direct count with a microscope is simple but tedious; more sensitive and accurate counts can be made with the aid of an electronic particle counter. More often, the turbidity of the culture is measured, because bacterial cultures above approximately $10^6$ cells/mL are visibly turbid, and turbidity is proportional to the total mass of bacterial protoplasm present per milliliter. Turbidity is quickly and easily measured by means of a spectrophotometer.

The growth rate of a bacterial culture depends on three factors: the species of bacterium, the chemical composition of the medium, and the temperature. The time needed for a culture to double its mass or cell number is in the range of 30 to 60 minutes for most pathogenic bacteria in rich media. Some species can double in 20 minutes (*E. coli* and related organisms), and some (eg, some mycobacteria) take almost as long as mammalian cells, 20 hours. In general, the greater the variety of nutrients provided in the medium, the faster growth occurs. This superficially simple fact actually depends on the operation of metabolic regulatory devices of considerable sophistication, which, as we shall see in the next section, ensures that building blocks provided in the environment not be wastefully synthesized by the cells. For each bacterial species, there is a characteristic optimum temperature for growth, and a range, sometimes as broad as 40°, within which growth is possible. Most pathogens of warm-blooded creatures have a temperature optimum for growth near normal body temperature, 37°C; growth often occurs at room temperature, but slowly. Therefore, incubators set at 35 to 37°C are used for culture of most clinical specimens. Exceptions to this rule include some organisms causing superficial infections for which 30°C is more suitable. As a group, bacteria have the widest span of possible growth temperatures, extending over the entire range of liquid water, 0°C to 100°C. Bacteria that grow best at refrigerator temperatures are called **psychrophiles,** those that grow above 50°C are called **thermophiles;** in between are the **mesophiles,** including virtually all pathogens.

Some species can divide every 20 minutes, others much more slowly

Growth rate is dependent on nutrient availability, pH, and temperature

Pathogens are mesophiles

When first inoculated, liquid cultures of bacteria characteristically exhibit a **lag period** during which growth is not detectable. This is the first phase of what is called the **culture growth cycle** (Fig 3–9). During this lag, the cells are actually quite active in adjusting the levels of vital cellular constituents necessary for growth in the new medium. Eventually net growth can be detected, and after a brief period of **accelerating growth,** the culture enters a phase of constant, maximal growth rate, called the **exponential** or **logarithmic phase** of growth, during which the generation time is constant. During this phase, cell number, and total cell mass, and amount of any given component of the cells increase at the same exponential rate; such growth is called **balanced growth,** or **steady-state growth.** The full reproductive potential of bacteria is exhibited during this phase: one cell gives rise to 2 cells in 1 generation, to 8 cells after 3 generations, to 1024 cells after 10 generations, and to about 1 million cells in 20 generations. For a bacterial species

Following a lag period, liquid cultures exhibit exponential growth during which generation time is constant and reproductive capacity enormous

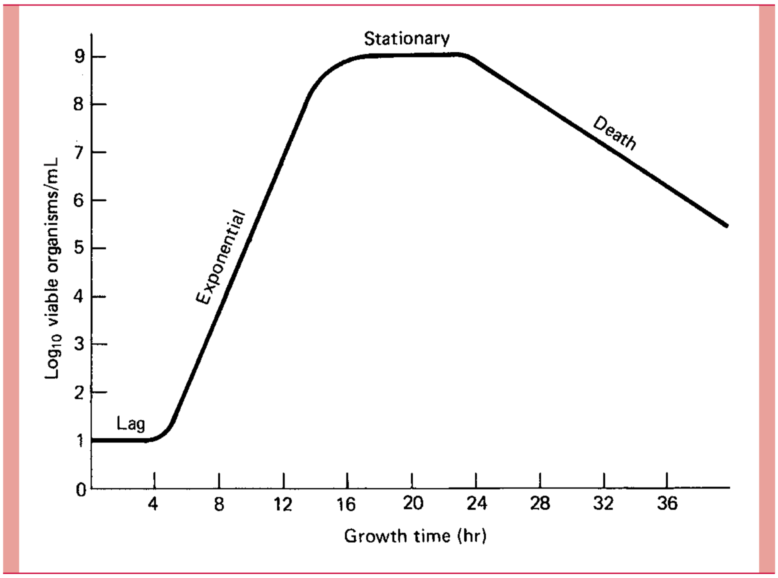


FIGURE 3–9
Phases of bacterial growth in liquid medium.

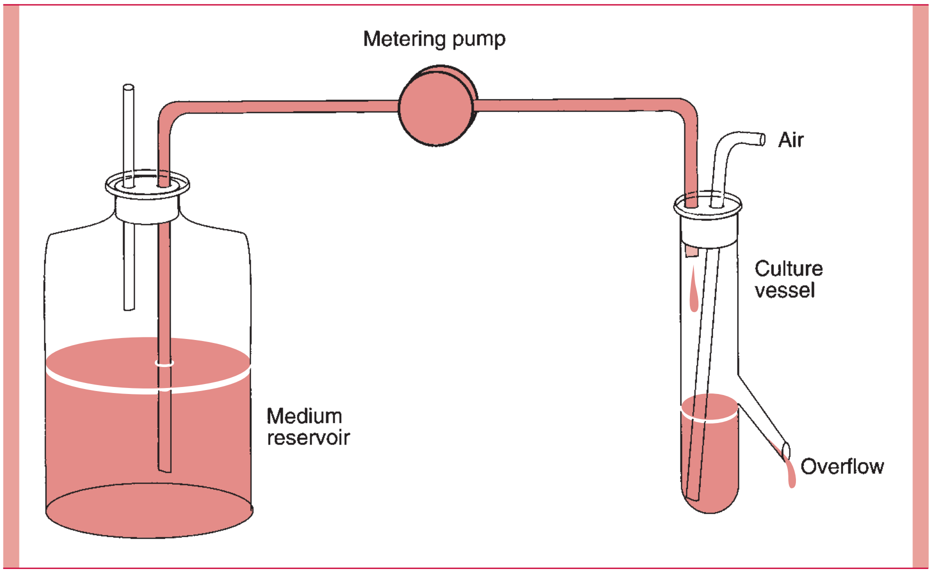


FIGURE 3–10
Schematic diagram of a chemostat. This continuous-culture device consists of a constant-volume growth chamber into which fresh sterile medium is fed at a constant rate by a pump.

with a generation time of 20 minutes, therefore, it takes less than 7 hours in the exponential phase of growth to produce a million cells from one.

Nutrient depletion or waste product accumulation terminates exponential growth

Cultures of some species die slowly after the stationary phase

By use of an equation for exponential growth, it can be demonstrated that 2 days of growth at this rate would be sufficient to generate a mass of bacteria equal to 500 times the mass of the earth. Fortunately, this never occurs, but not because the equation is faulty. Constant growth rate requires that there be no change in the supply of nutrients or the concentration of toxic by-products of metabolism (such as organic acids). This constancy can exist for only a short time (hours) in an ordinary culture vessel. Then growth becomes progressively limited (**decelerating phase**) and eventually stops (**stationary phase**). Cells in the stationary phase are different from those in the exponential phase. They are smaller, have a different complement of enzymes (to deal with survival during starvation), and have fewer ribosomes per unit mass. When an inoculum of such cells is placed into fresh medium, exponential growth cannot resume immediately, and hence the lag period is observed. Note that there is no lag phase if the inoculum consists of exponential-phase cells. Prolonged incubation of a stationary-phase culture leads to cell death for many bacterial species (such as the pneumococcus), although many (such as *E. coli*) are hardy enough to remain viable for days. During the **death phase** or **decline** of a culture, cell viability is lost by exponential kinetics as described in Chapter 11. As already noted, for those Gram-positive species that can sporulate, entry into the stationary phase usually triggers this event.

Continuous exponential growth can be maintained in a chemostat or a turbidostat

One way to maintain a culture in exponential, steady-state (balanced) growth for long periods is to use a device in which fresh medium is continuously added but the total volume of culture is held constant by an overflow tube. One such constant-volume device is called a **chemostat;** it operates by infusing fresh medium containing a limiting nutrient at a constant rate, and the growth rate of the cells is set by the flow rate (Fig 3–10). A similar constant-volume device is the **turbidostat;** it operates by the infusion of fresh medium by a pump controlled indirectly by the turbidity of the culture. Although such devices may sound artificial, they mimic many situations of interest to medical microbiologists. Most of the places in which bacteria live on and within our bodies, in health and disease, provide conditions more closely resembling those of nutrient-limited continuous-culture devices than of enclosed flasks.

## BIOFILMS

Except for growth as colonies on agar-solidified media, bacterial cultures grown in a laboratory are smooth suspensions of individual cells dispersed in a liquid medium (see Chapter 15). In nature, whether in soil, in marine or riparian environments, or on the surface of physical agents, including medical prosthetic devices, bacteria grow as aggregated assemblies of cells. These **biofilms** frequently develop a multicellular arrangement that excludes antimicrobics and other toxic molecules and enhances the ability of the bacteria to capture nutrients. The full extent to which this phenomenon is related to infectious disease remains to be determined, but it is clear that adherence to cell and tissue surfaces is an attribute of most pathogens.

## REGULATION AND ADAPTATION

Metabolic reactions must proceed in a coordinated fashion. It would not do to have them governed solely by the laws of "mass action" by which the concentrations of reactants and products determine the rate of reactions. Furthermore, it would not do to have rates of individual reactions set at some fixed levels. Bacteria can do little to control their environment, and any change in environment (eg, in temperature, pH, nutrient availability, osmolarity) would disrupt any preset synchronization or render it inappropriate. Bacteria must, therefore, not just coordinate reactions, but must do so in a flexible, adjustable manner to make growth possible in a changing environment. They accomplish this feat by many regulatory mechanisms, some of which operate to control **enzyme activity,** some to control **gene expression.**

Flexible coordination of metabolic reactions occurs by regulating both enzyme activity and gene expression

### Control of Enzyme Activity

Although there are many examples of covalent modification of enzymes (eg, by phosphorylation, methylation, or acylation) to alter their activity, by far the most prevalent means by which bacterial cells modulate the flow of material through fueling and biosynthetic pathways is by changing the activity of **allosteric enzymes** through the reversible binding of low-molecular-weight metabolites **(ligands).** In fueling pathways it is common for AMP, ADP, and ATP to control the activity of enzymes by causing conformational changes of allosteric enzymes, usually located at critical branch points where pathways intersect. By this means, the flow of carbon from the major substrates through the various pathways is adjusted to be appropriate to the demands of biosynthesis. For example, the **energy charge** of the cell, defined as (ATP + 1/2 ADP)/(ATP + ADP + AMP), is kept very close to 0.85 under all conditions of growth and nongrowth. In biosynthetic pathways, it is common for the end product of the pathway to control the activity of the first enzyme in the pathway. This pattern, called **feedback inhibition** or **end-product inhibition,** ensures that each building block is made at exactly the rate it is being used for polymerization. It also ensures that building blocks supplied in the medium are not wastefully duplicated by synthesis. Because many biosynthetic pathways are branched and have multiple end products, special arrangements must be made to produce effective regulation. These include the production of multiple isofunctional enzymes for the controlled step, the design of allosteric enzymes that require the cumulative effect of all end products to be completely inhibited, and sequential inhibition of each subpathway by its last product (Fig 3–11).

Most metabolic pathways are controlled by allosteric enzymes

Fueling pathway enzymes are controlled by AMP, ADP, and ATP concentrations to maintain energy charge

Feedback inhibition controls biosynthetic pathways for both economy and efficiency

### Control of Gene Expression

To a far greater extent than eukaryotic cells, bacteria regulate their metabolism by changing the amounts of different enzymes. This is accomplished chiefly by governing their rates of synthesis, that is, by controlling gene expression. This works rapidly for bacteria because of their speed of growth; shutting off the synthesis of a particular enzyme results in short order in the reduction of its cellular level due to dilution by the growth of the cell. Most importantly, bacterial mRNA is degraded rapidly. With an average half-life of 2 to 3 minutes at 37°C, the mRNA complement of the cell can be totally changed in a small fraction of a generation time. The synthesis of a given enzyme can therefore be rapidly turned on and just as rapidly turned off simply by changes in the rate of transcription of its gene.

Changes in transcription can rapidly change enzyme synthesis because of mRNA degradation

Most, although not all, of the regulation of gene expression occurs at or near the beginning of the process: the initiation of transcription. That is, gene expression is not

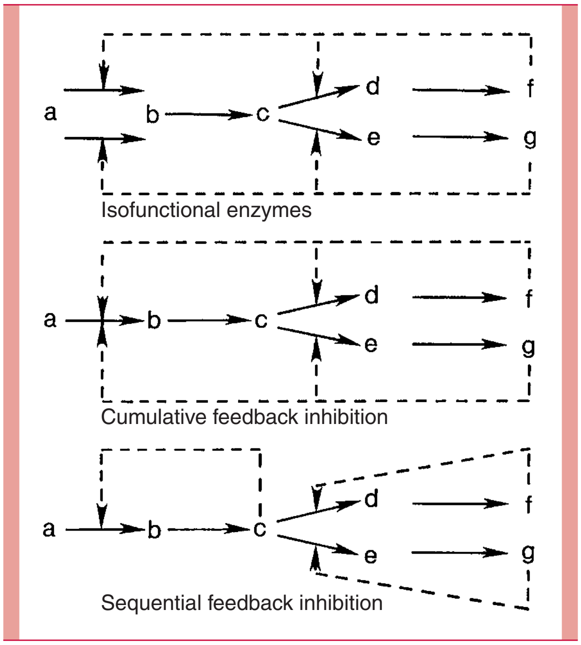


FIGURE 3–11
Patterns of end-product inhibition in branched biosynthesis pathways.

Much regulation operates at initiation of transcription

regulated by changing the rate of mRNA chain elongation; once started, transcription proceeds at a more or less constant rate. Regulation occurs by a decision of whether to initiate or not, or what amounts to the same thing, by setting the frequency of initiation.

Bacterial genes are organized as transcriptional units called operons, each of which contains one or more cistrons that encode polypeptides

RNA polymerase binds to the promoter of an operon and transcribes until it meets the terminator

A closer look at transcription is necessary to understand how it is controlled. Most of the genes we know about in bacteria are organized as **multicistronic operons.** A **cistron** is a segment of DNA encoding a polypeptide. An **operon** is the unit of transcription; the cistrons that it comprises are cotranscribed as a single mRNA. The structure of a typical operon (Fig 3–12) consists of a **promoter** region, an **operator** region, component cistrons, and a **terminator.** In the best-studied bacterium, *E. coli,* RNA polymerase, programmed by the major replaceable $\sigma$ subunit, $\sigma$-70, recognizes the promoter region and binds to the DNA. Initially the binding is a closed complex, but this can be converted into an open complex in which the two strands of DNA are partially separated. Strand separation exposes the nucleotide bases and permits initiation of synthesis of a mRNA strand complementary to the sense strand of the DNA. In a simple case, transcription continues through the cistrons of the operon until the termination signal is reached. In some cases recognition of the termination signal requires another removable subunit of RNA polymerase, $\rho$. This process is shown in Figure 3–12.

Activator and repressor proteins regulate transcription by binding to the operator region of operons

Near the promoter in many operons is an operator to which a specific **regulator protein** or **transcription factor** can bind. In some cases the binding of this regulator blocks initiation; in such a case of negative control, the regulator is called a **repressor.** Repressors are allosteric proteins, and their binding to the operator depends on their conformation, which is determined by the binding of ligands that are called **corepressors** if their action permits binding of the repressor and **inducers** if their action prevents binding. In some cases, the regulator protein is required for initiation of transcription, and it is then called an **activator.** The functioning of both types of regulator proteins on transcription initiation is illustrated in Figure 3–13 using the regulation of the *lac* operon as an example. This operon encodes proteins necessary for the use of lactose as a carbon and energy source.

Some proteins regulate transcription by binding to enhancers and bending DNA

Some regulator proteins bend DNA on binding, and this can bring together what would otherwise be distant sites of the DNA. In this manner, proteins bound at sites called **enhancers** far upstream or downstream of a promoter can be brought into physical contact with RNA polymerase and influence its activity. One such DNA bender in ***E. coli*** is called the **integration host factor.**

Many regulator proteins are converted from inactive to active forms by covalent modification rather than by the allosteric binding of a ligand. Phosphorylation is by far the

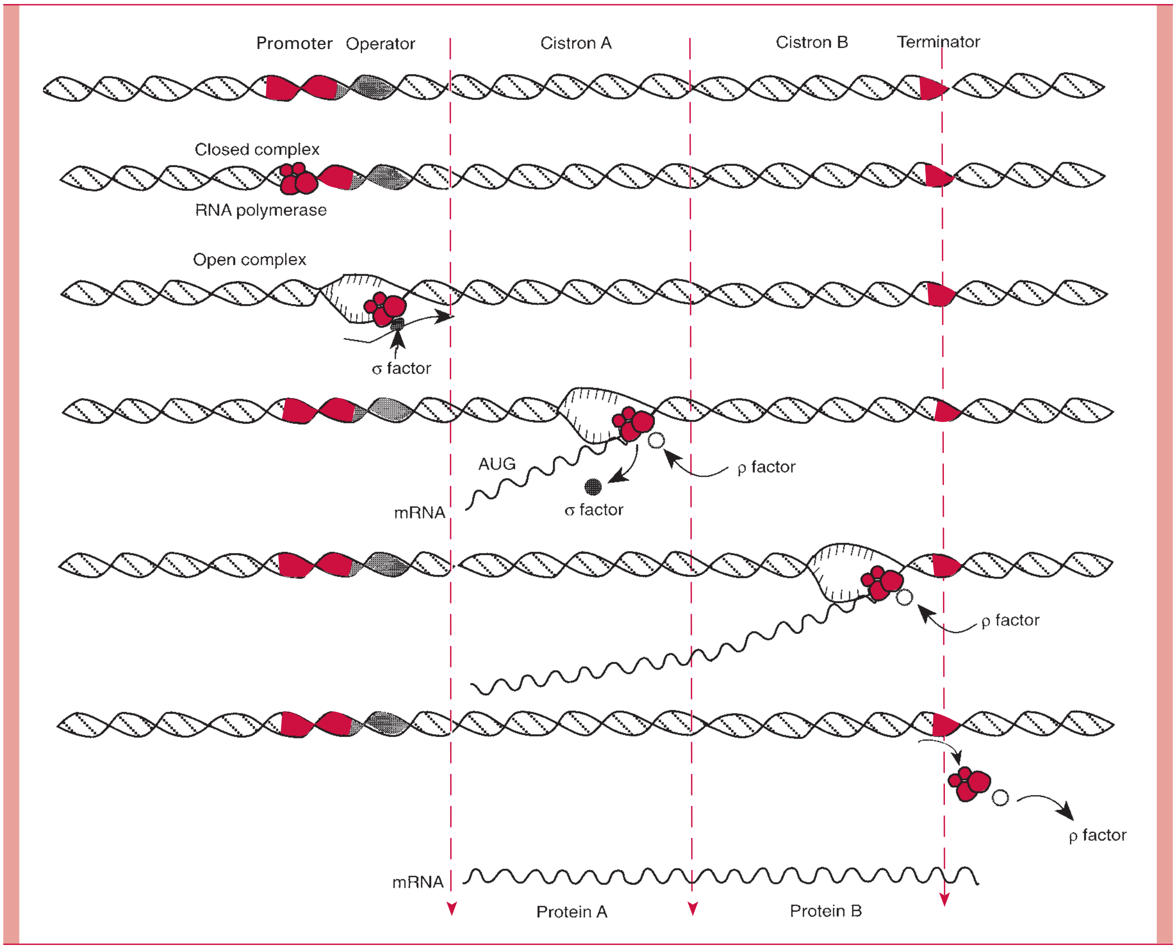


FIGURE 3–12
Control of transcription. Schematic representation of a bacterial operon and its transcription by RNA polymerase.

most common modifying event and operates in the widespread two-component **signal transduction** pathways described below under cell stress regulons.

Some regulators are controlled by phosphorylation

Once transcription is initiated it may continue uneventfully, but in some operons another site of control is quickly encountered. After transcription of a **leader region,** the RNA polymerase encounters a region known as an **attenuator.** Synthesis of mRNA is aborted at the attenuator; only a small percentage of the RNA polymerase molecules reaching the attenuator can successfully pass through it. However, the activity of the attenuator can be modified by a process that involves not a regulator protein but rather changes in the secondary structure of the mRNA. This regulatory process is illustrated in Figure 3–14 using the *his* operon, which encodes the enzymes necessary for the biosynthesis of the amino acid L-histidine, as an example. In enteric bacteria, attenuation is a common means of controlling biosynthetic operons. Note that it differs from the repression mechanism in that it requires no special regulatory gene or regulatory proteins.

Attenuation regulates some biosynthetic operons by controlling abortion of transcription early in the operon

There are many instances known in which groups of genes that are independently controlled as members of different operons must cooperate to accomplish some response to an environmental change. When such a group of genes is subject to the control of a common regulator, the group is called a **regulon.** One such regulon, or **global control system,** is catabolite repression. Its function is to prevent the cell from responding to the presence of alternative carbon sources when the environment already provides a more than adequate supply from the preferred substrate, glucose. This control is brought about as follows. Operons that encode catabolic enzymes (those responsible for initiating the use of carbon sources, such as lactose, maltose, arabinose, and other sugars and amino

Regulons are groups of unlinked operons controlled by a common regulator

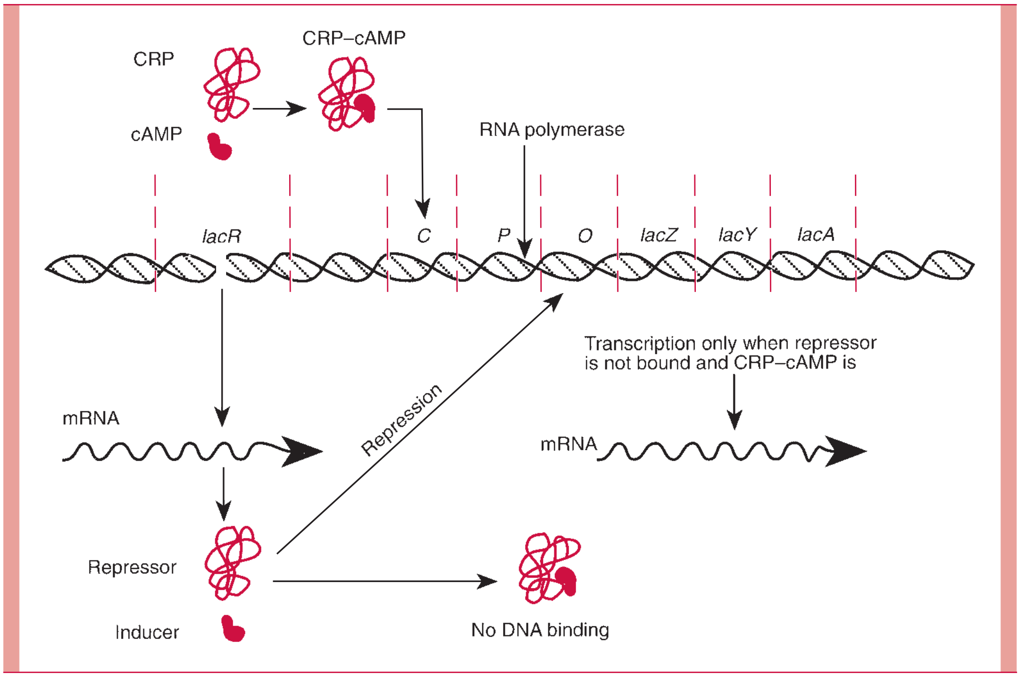


FIGURE 3–13
Schematic representation of the control of transcription initiation by repressor and activator proteins. The example chosen is the *lac* operon of *Escherichia coli. LacR* (or I), gene encoding the lac repressor protein; C, CAP region (binding site of cAMP receptor protein, or CRP); P, promoter region (binding site of RNA polymerase); O, operator region (binding site of repressor); *lacZ,* gene encoding ß-galactosidase; *lacY,* gene encoding permease for $\beta$-galactosides; *lacA,* gene encoding galactoside acetylase.

acids) have weak promoters that need help to promote high-level initiation of transcription by RNA polymerase. The help is supplied by a regulator protein called **catabolite activator protein** or **cAMP receptor protein** (CRP). This protein, if and only if cyclic AMP is bound to it, binds slightly upstream from the promoter and permits high-level expression if the operon is specifically induced (and the repressor has been removed by induction). Because cAMP levels are very low during growth on glucose or other favored substrates, there is insufficient cAMP–CRP complex to activate catabolic operons even if their inducers are present in the environment. As a result, the cells ignore the induction signal if they have an adequate supply of glucose.

Catabolite repression regulon ensures optimal use of preferred substrates

Finally, gene regulation in bacteria is accomplished by unique tactics so far discovered only in pathogens. These are included in the following section.

## CELL SURVIVAL

### Cell Stress Regulons

From studies with *E. coli,* it was learned that cells have many regulons involved in survival responses during difficult circumstances. The catabolite repression regulon just described is in essence a means by which the cell can optimize its synthesis of catabolic enzymes by making only those that contribute to growth. But this regulon can also be viewed as a survival device, helping the cell to respond to the nutritional stress of running out of glucose. If an alternative source of carbon is present in the environment, the cell can redirect its pattern of gene expression to make a suitable adjustment to the nutritional stress.

Bacteria have regulons that help cope with environmental stress

SOS system repairs damaged DNA and prevents multiplication during repair

Perhaps more obvious as a stress response is the **SOS system,** a set of 17 genes that are turned on when the cell suffers damage to its DNA. The products of these genes are

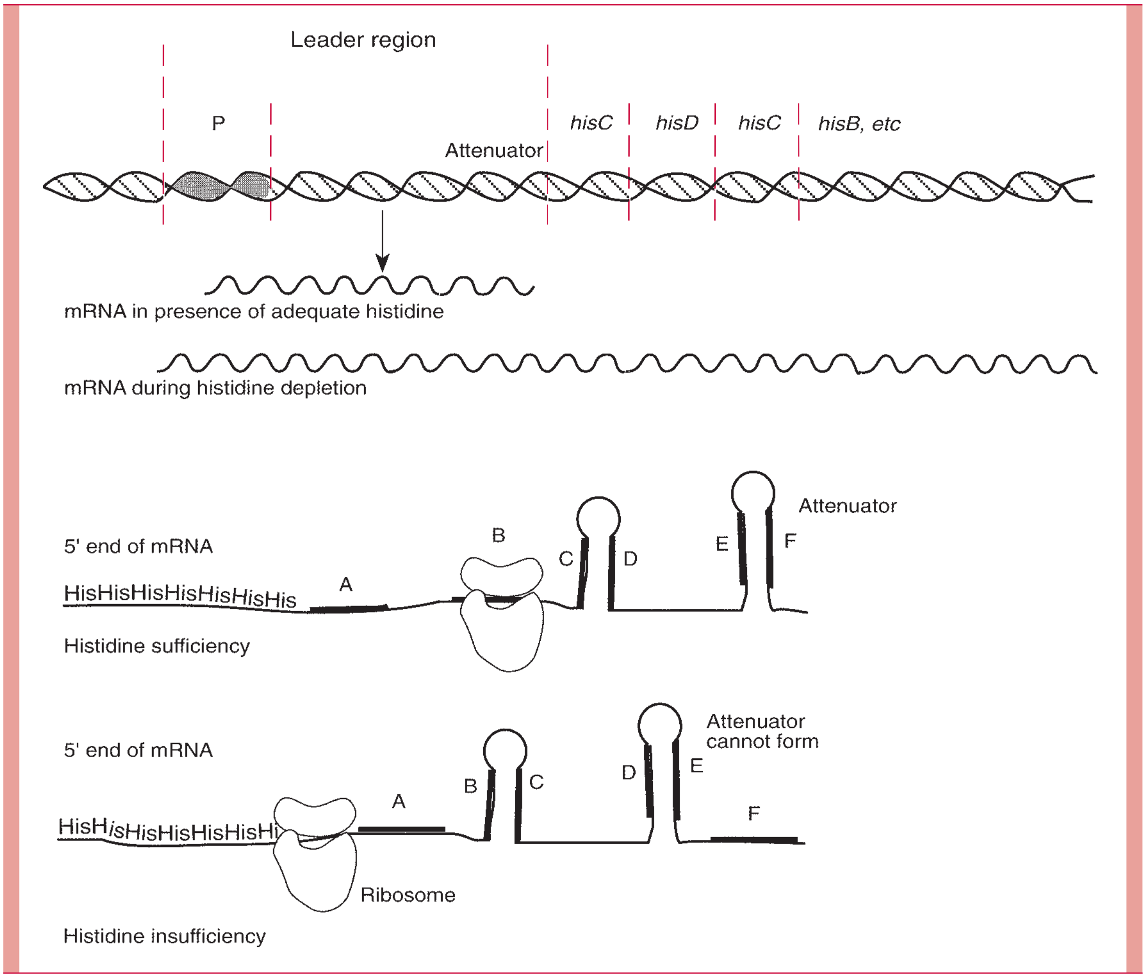


FIGURE 3–14
Schematic representation of the control of transcription by the process of attenuation. The example chosen is the *his* operon of *Escherichia coli*. How attenuation works is fascinating. The leader region is always transcribed and translated into a small oligopeptide. The peptide near the attenuator site has a string of seven *his* codons. Movement of the first ribosome coming behind the polymerase is drastically affected by the supply of charged *his* tRNA. If there is an adequate supply, the ribosome is not delayed, and an attenuator loop forms in the mRNA, causing transcription to terminate. With a shortage of histidine, the first ribosome gets hung up over the *his* codons, and the attenuator loop is not formed, because alternate loops form. As a result, transcription proceeds, the complete *his* mRNA is made, and the biosynthetic enzymes can be made in large quantities. The upper portion of the figure illustrates the difference in transcription of the *his* operon in histidine sufficiency and insufficiency; the lower two diagrams depict the molecular mechanism of attenuation.

involved in several processes that repair damaged DNA and prevent cell division during the repair.

Another prominent bacterial cell stress regulon is responsible for the **heat-shock response.** It encompasses some 20 genes, which are transcriptionally activated on an upward shift in temperature or on imposition of several kinds of chemical stress, including alcohol. In the case of *E. coli,* the heat-shock regulator protein is a special subunit of RNA polymerase, $\sigma$-32, which replaces the normal $\sigma$-70 subunit and locates the special promoters of the heat-shock genes. At least half of the heat-shock genes encode proteins that either are proteases or are **protein chaperones** that assist in the processing, maturation, or export of other proteins. It is thought that these chaperones and proteases are needed for normal protein processing at all temperatures but are required in higher amounts to counteract the

Heat-shock gene expression is enhanced at high temperature and allows cell survival

Some heat-shock genes encode protein chaperones effects of high temperature on protein folding and protein–protein interactions. The bacterial chaperones are highly similar to their mammalian counterparts. For example, HtpG, DnaK, and GroEL of *E. coli* correspond to the mammalian hsp90, hsp70, and hsp60 families of chaperones, respectively. The precise involvement of the heat-shock response in infectious disease is still being explored, but it is striking that antibodies directed against bacterial heat-shock proteins constitute a major component of the serologic response of humans to infection or vaccine administration. Fever in humans can elevate body temperature sufficiently to induce the heat-shock response, and it is suspected that this response may affect the outcome of various infections. Also, some viruses both of bacteria and of humans use the heat-shock proteins of their host cells to promote their own replication.

Response to environment involves phosphorylation of a pair of specific signal transduction proteins, a protein kinase, and a response regulator

Other regulons deal with cell survival in the face of such stresses as osmotic shock, high or low pH, oxidation damage, presence of toxic metal ions, and restrictions for fundamental nutrients (phosphate, nitrogen, sulfur, and carbon). A large number of these responses involve teams of proteins that sense the environment, generate a signal, transmit that signal by protein–protein interactions, and activate the appropriate response regulon. In a striking number of cases, a response system includes a **protein kinase** that becomes phosphorylated by ATP on a particular conserved histidine residue in response to an environmental stimulus. This kinase is teamed with a second protein called a **phosphorylated response regulator.** The phosphate residue from the kinase is transferred to an aspartic acid residue of the response regulator, usually converting this protein into an activator of transcription of the appropriate genes. Members of these two families of **signal transduction proteins** share highly conserved domains throughout distantly related bacteria.

## Endospores

Sporulation involves sequential activation of interrelated regulons resulting in production of a resistant endospore, capable later of germination

Two of the most elaborate bacterial survival responses involve the transition of growing cells into a form that can survive long periods without growth. In a few Gram-positive bacterial species, this involves **sporulation,** the production of an **endospore,** as we saw in Chapter 2. This process, extensively studied in a few species, involves cascades of RNA polymerase $\sigma$ subunits, each sequentially activating several interrelated regulons that cooperate to produce the elaborately encased spore, which though metabolically inert and extremely resistant to environmental stress, is capable of germinating into a growing (vegetative) cell.

## Stationary Phase Cells

Formation of a stationary phase cell involves activation of many regulons in a coordinated cascade

For all other bacteria, adaptation to a nongrowing state involves formation of a differentiated cell called the **stationary phase cell.** The product is certainly far different morphologically from an endospore, but a tough, resistant, and metabolically quiescent cell is produced that looks distinct from its growing counterpart. Its envelope is made tougher by many modification of its structure, its chromosome is aggregated, and its metabolism is adjusted to a maintenance mode. Producing this resistance involves a process surprisingly analogous to sporulation, because, as in sporulation, cascades of signals and responses involving the sequential activation of sets of genes appear to be involved. One of the many global regulators involved is RpoS, a $\sigma$ subunit of RNA polymerase.

## Motility and Chemotaxis

Flagellar motor uses proton-motive force energy

Motility in most bacterial species is the property of swimming by means of flagellar propulsion. The complex structure of a flagellum—its filament, hook, and basal body—was presented in Chapter 2. The helical filament functions as a propeller, the hook possibly as a universal joint, and the basal body with its rod and rings as a motor anchored in the envelope. The flagellar motors turn the filaments using energy directly from the electrochemical gradient (proton-motive force) of the cell membrane rather than from ATP. The filament can be rotated either clockwise or counterclockwise. Whatever the number of flagella on a cell and whatever their arrangement on the surface (polar, peritrichous, or lophotrichous), they are synchronized to rotate simultaneously in the same direction. Only counterclockwise rotation results in productive vectorial motion, called a **run.** Clockwise rotation of the flagella causes the cell to **tumble** in place. The flagella alternate between periods of clockwise and counterclockwise rotation according to an endogenous schedule. As a result, motile bacteria move in brief runs interrupted by periods of tumbling.

Direction of flagellar rotation determines a run or a tumble

**Chemotaxis** is directed movement toward chemical **attractants** and away from chemical **repellents.** It is accomplished by a remarkable molecular sensory system that possesses many of the characteristics that would be expected of behavioral systems in higher animals, including memory and adaptation. Beside the genes of the flagellar proteins (called *fla,* for flagella) more than 30 genes (called *mot,* for motility, and *che,* for chemotaxis) encode the proteins that make this system work: receptors, signalers, transducers, tumble regulators, and motors.

Multiple genes are required for chemotaxis

Whether a cell is moving toward an attractant or away from a repellent, chemotaxis is achieved by **biased random walks.** These result from alterations in the frequency of tumbling. When a cell is, by chance, progressing toward an attractant, tumbling is suppressed and the run is long; if it is swimming away, tumbling occurs sooner and the run is brief. It is sheer chance in what direction a cell is pointed at the end of a tumble, but by regulating the frequency of tumbles in this manner, directed progress is made.

Changes in duration of runs and tumbles determine chemotactic response

The mechanism of chemotaxis is fairly well understood from work with *E. coli*. It is complex and can be summarized as follows. Binding of an attractant alters the endogenous routine schedule of runs and tumbles by interrupting a **phosphorylation cascade** and thus prolonging the run. Accommodation by a **methylation** system restores the endogenous schedule and resets the cell's sensitivity to the attractant to require a higher concentration to prolong the run. This constitutes a **molecular memory.** The bacterial cell senses a concentration gradient not by measuring a difference between the concentration at each end of the cell but by a molecular memory that enables it to compare the concentration now with what it was a short time ago. Escape from a repellent occurs in an analogous fashion.

Molecular memory recognizes change in attractant concentration and ensures progress toward it

Chemotaxis is both a survival device (for avoiding toxic substances) and a growth-promoting device (for finding food). It can also be a virulence factor in facilitating colonization of the human host by bacteria.

Chemotaxis serves survival, growth-promoting, and pathogenic roles

## BACTERIAL VIRULENCE

### Special Attributes of Pathogens

Most bacteria have the ability to grow and survive under harsh conditions. Yet of the many thousands of bacterial species, only a small percentage are associated with humans as part of the natural flora or as causative agents of disease. This fact generates the question central to medical microbiology from the very start: What makes a bacterium pathogenic? The answer is not simple, because it turns out that many properties are necessary for a bacterial cell to gain entrance to a human, evade its defense systems, and establish an infection. The structures and activities described in Chapter 2 and in this chapter bear directly on virulence attributes of bacteria. They include:

Virulence results from many specialized bacterial structures and activities

- Adherence to and penetration of host cell surfaces
- Evasion of phagocytic and immunologic attack
- Secretion of toxic proteins to weaken the host and promote spread of the pathogen
- Acquisition of nutrients, including iron, to permit growth within the host
- Survival under adverse conditions both within and outside the host and its macrophages

As we shall see in detail in the next chapter, the genes unique to pathogens are frequently found clustered in genetic segments within either plasmids or the bacterial chromosome, with interesting implications for the evolution of pathogenic bacteria. These subjects are examined in detail in Chapter 10.

### Regulation of Virulence

Most bacterial pathogens must survive and grow in two very different circumstances—in the broad external environment and in or on the human host. Expressing the very

Virulence genes must be regulated

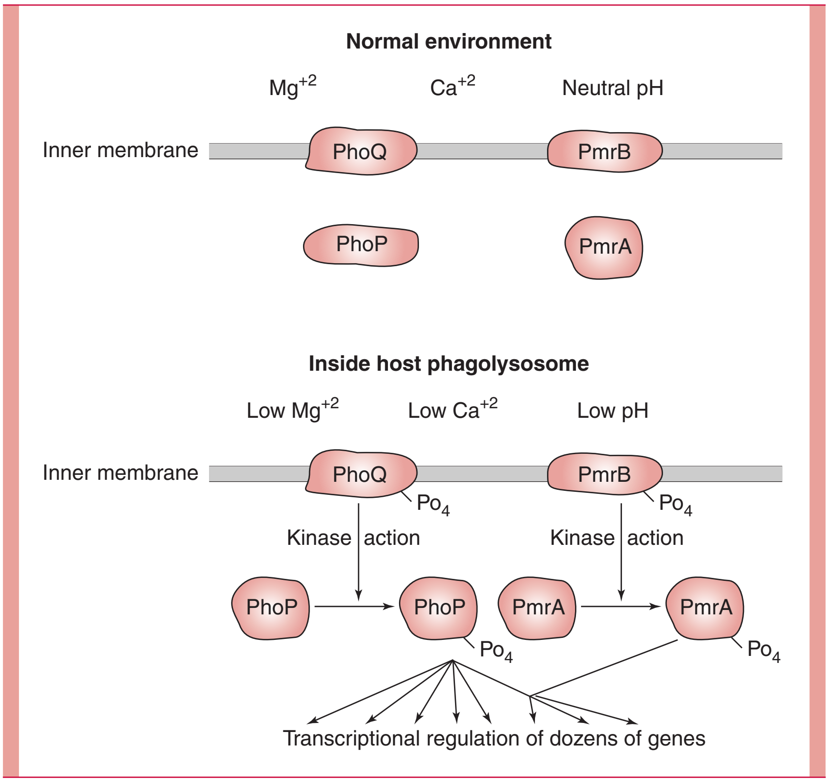


FIGURE 3–15
Schematic representation of regulation of virulence genes by the PhoP/PhoQ system of *Salmonella*. (*Adapted from Harper JR, Silhavy TJ. Germ warfare: The mechanisms of virulence factor delivery. In: EA Groisman,* Principles of Bacterial Pathogenesis, *San Diego, CA: Academic Press, 2001, pp 43–74.*)

many genes responsible for pathogenesis in the latter situation would be counterproductive, if not outright detrimental to growth and survival in nature, while failing to express them on encountering the host would commonly be fatal. To ensure their growth and survival in both circumstances, bacteria have evolved elaborate and effective mechanisms for regulation of virulence genes.

Many virulence genes are part of conventional response regulons

Virulence genes are commonly organized as regulons, and many of these share the attributes of general stress response regulons, described above. For example, many are regulated by classical two-component signal transcription systems in which environmental sensing is achieved by a sensor protein kinase, which relays information about the environment by phosphorylating its partner, a response regulator, which in turn acts to control transcription initiation of its gene set. Many dozens of these systems have been found in various pathogens. Here we examine one example, the **PhoP/PhoQ system,** which is essential to the virulence of *Salmonella* (Fig 3–15). PhoQ is a sensor protein kinase in the cell membrane, and PhoP is the response regulator to which it relates. PhoQ is sensitive to the concentration of magnesium ion in the periplasm of the *Salmonella* cell. With a normally adequate $Mg^{2+}$ concentration, PhoQ is locked in an inactive state; however, when the concentration is very low, as happens when the *Salmonella* finds itself within the phagolysosome of human macrophages, PhoQ autophosphorylates one of its histidine residues. Phosphorylation of PhoP ensues, and this event activates it as a transcription regulator. The phosphorylated PhoP controls more than 40 genes. Some are induced, including those encoding an acid phosphatase, cation transporters, outer membrane proteins, and enzymes that modify LPS. Some are repressed, including some encoding proteins essential for epithelial cell invasion and others encoding components of a contact secretion system. The control network is complex, because some of the regulated genes are not directly controlled by PhoP, but by a second two-component response system, called PmrA/PmrB, which is responsive to low pH. The two systems, PhoP/PhoQ and PmrA/PmrB, act in cascade fashion to accomplish a rather intricate response. The induced proteins are believed to enable the cell to scavenge $Mg^{2+}$ from its own LPS and to protect itself from the hostile environment of the phagolysosome; the repressed ones were useful in earlier stages of the infective process but now are superfluous. The *Salmonella* cell that finds itself in the phagolysosome of a macrophage has evolved a way to sense its situation and maximize its production of needed factors while dispensing with irrelevant ones.

An interesting principle has emerged from the study of bacterial luminescence and from the field of infectious diseases of plants. Researchers have discovered that several types of bacteria, including *Pseudomonas,* regulate the expression of genes in a cell density–dependent manner. That is, expression of certain genes occurred only when the population density of the bacteria reached a threshold level, called a **quorum.** Quorum sensing in some cases is achieved by secretion of a small, diffusible molecule (some are acyl-homoserine lactones) that is sensed by an envelope protein, triggering a regulatory response through a two-component signal transduction system. This **autoinduction** enables the bacteria to avoid "tipping their hand" and mounting an attack on the host before their numbers are sufficient to overwhelm the host's defenses. *Salmonella* are known to have a quorum-sensing protein, SdiA, that regulates at least one operon on a virulence plasmid in these cells. No evidence yet indicates the role of this regulation in *Salmonella* infection. Exploration of the possible role of quorum sensing in general in human disease is ongoing.

Some genes are autoinduced by cells after a critical population density, or quorum, is reached

Regulation of virulence gene expression is achieved also in a fashion totally unexpected from the study of metabolic gene regulation, namely by rearrangement of DNA. These instances do not involve mutations in the usual sense of the term, because the DNA alterations are readily reversible. Many well studied examples have generated the concept of a genetic switch, with an "on" and an "off" position determined by the inversion of a small segment of DNA adjacent to the regulated genes. Examples of this and related mechanisms dependent on DNA recombination are presented in Chapter 4.

Some virulence genes are controlled by genetic switches and other DNA rearrangements

## ADDITIONAL READING

Groisman EA. *Principles of Bacterial Pathogenesis*. San Diego, CA: Academic Press; 2001. Chapter 2 (Harper JR, Silhavy TJ), Germ Warfare: The Mechanisms of Virulence Factor Delivery, presents details of protein export. Chapter 3 (Dorman CJ, Smith SGJ), Regulation of Virulence Gene Expression in Bacterial Pathogens, is a comprehensive description of the myriad of molecular mechanisms used by pathogens to regulate virulence genes.

Neidhardt FC, Ingraham JL, Schaechter M. *Physiology of the Bacterial Cell: A Molecular Approach.* Sunderland, MA: Sinauer Associates; 1990. Chapters 3 through 8 present bacterial metabolism and physiology in a manner similar to what was done here, but in more detail.

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CHAPTER 4

# Bacterial Genetics

FREDERICK C. NEIDHARDT

No aspect of the basic biology of the prokaryotic cell is so unfamiliar outside the community of microbiologists as the genetics of these essentially asexually reproducing cells. And yet, this subject has extremely important practical messages for clinicians and others interested in infectious disease. The genetic determinants of microbial properties and the rules that determine microbial evolution are of paramount importance to the treatment regimen for an individual patient as well as to thoughts about the origin and future course of the human–microbe interaction. This chapter explores the fundamentals of this area.

## BACTERIAL VARIATION AND INHERITANCE

It was rather difficult to establish that many of the same rules of heredity apply to bacteria as to plants and animals. This may seem strange, because the most spectacular advances in molecular genetics have been achieved almost exclusively through work on *Escherichia coli* and its viruses. However, during the 1940s and early 1950s, serious experimental efforts were still being directed toward determining whether mutations in bacteria were random or specifically directed by the environment.

The difficulty of establishing the basis of heredity in bacteria grew out of their inherent properties and their manner of growth. First, because bacteria are **haploid,** the consequences of a mutation, even a recessive one, are immediately evident in the mutant cell. Because the generation time of bacteria is short, it does not take many hours for a mutant cell that has arisen by chance to become the dominant cell type in a culture under appropriate selective conditions. This can lead to the false conclusion that the environment has directed a genetic change. Second, as was noted in Chapter 3, bacteria, to a far greater extent than animals and plants, respond to change in their chemical and physical environment by altering their pattern of gene function, thereby taking on previously unexpressed properties. For example, *E. coli* cells make the enzymes for lactose metabolism only when grown with this sugar as carbon source. Superficially this might suggest that lactose changes the cell's **genotype** (its complement of genes), when instead it is only the **phenotype** (the characteristics actually displayed by the cell) that has been changed by the environment. Finally, even when rather exceptional technical measures are taken to ensure a pure culture, contamination can occasionally occur. With cultures containing more than one bacterial species, different conditions of growth can cause one or another species to predominate (by, for example, a million to one ratio), suggesting to the unwary observer that the characteristics of "the" bacterium under study are very unstable and dependent on the environment.

Mutations are rapidly expressed; mutants quickly predominate under selective conditions

Environment can influence phenotypic expression of genotype

Progress in bacterial genetics was rapid once it was recognized that mutation and selection can quickly change the makeup of a growing population and that bacterial cells inherit genes that may or may not be expressed depending on the environment. Even so, it

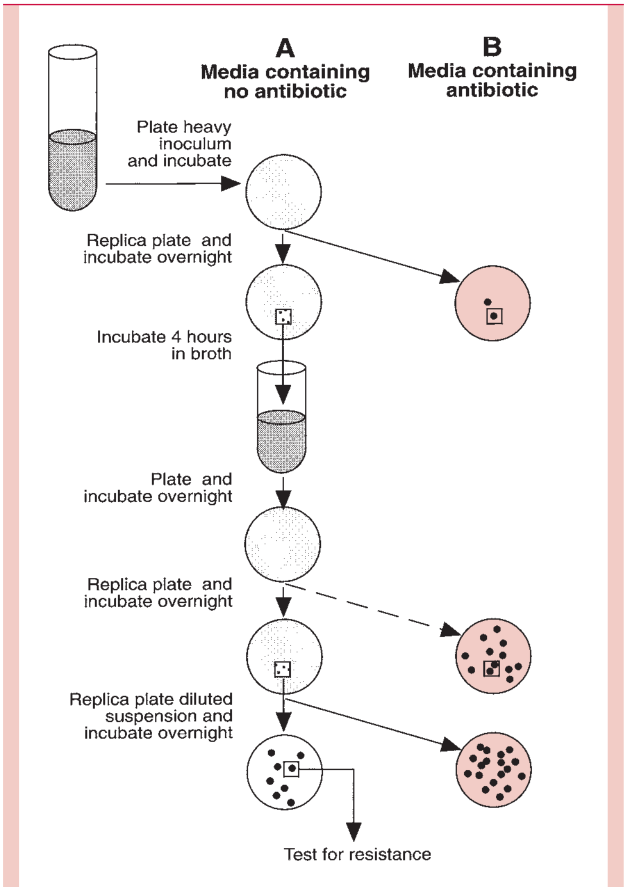


FIGURE 4–1
Lederberg technique for indirect selection of antimicrobic resistant mutants. Growth on plates in the left-hand column **(A)** is replicated to antimicrobic-containing plates in the right-hand column **(B)**. If resistant mutants arise in the absence of antimicrobic **(A)**, the position of colonies on antimicrobic-containing plates would indicate their position on the plates that do not contain antimicrobic. By selecting growth from this position and repeating the process with appropriate inoculum dilutions, resistant mutants that have never been exposed to the antimicrobic can be directly selected **(A)**.

Novel agents that transfer genes account for many puzzling genetic events

was not until the discovery in the 1980s of transposable genetic elements and insertion sequences (to be discussed later in this chapter) that certain examples of high-frequency variation, the so-called **phase transition,** could be satisfactorily explained within the framework of classic genetic principles.

Proof of randomness of mutation was important in clinical medicine

Several experiments were particularly important in establishing that mutations occur in nature as random events and are not guided by the environment. The most convincing introduced the technique of replica plating and was used to show how a population of cells totally resistant to an antimicrobic could be isolated from an initially sensitive population without ever exposing them to the toxic agent (Fig 4–1). This clarified the mechanism of an important clinical problem.

## MUTATION AND REPAIR

The spontaneous development of mutations is a major factor in the evolution of bacteria. Mutations occur in nature at a low frequency, on the order of one mutation in every million cells for any one gene, but the large size of microbial populations ensures the presence of many mutants.

### Kinds of Mutations

The several kinds of mutations all involve changes in nucleotide sequence

**Mutations** are heritable changes in the structure of genes. The normal, usually active, form of a gene is called the **wild-type allele;** the mutated, usually inactive, form is called the **mutant allele.** There are several kinds of mutations, based on the nature of the change in nucleotide sequence of the affected gene(s). **Replacements** involve the substitution of one base for another. **Microdeletions** and **microinsertions** involve the removal and addition, respectively, of a single nucleotide (and its complement in the opposite strand). **Insertions** involve the addition of many base pairs of nucleotides at a single site. **Deletions** remove a contiguous segment of many base pairs. **Inversions** change the direction of a segment of

DNA by splicing each strand of the segment into the complementary strand. **Duplications** produce a redundant segment of DNA, usually adjacent (tandem) to the original segment.

By recalling the nature of genes and how their nucleotide sequence directs the synthesis of proteins, one can understand the immediate consequence of each of these biochemical changes. If a replacement mutation in a codon changes the mRNA transcript to a different amino acid, it is called a **missense mutation** (eg, an AAG [lysine] to a GAG [glutamate]). The resulting protein may be enzymatically inactive or very sensitive to environmental conditions, such as temperature. If the replacement changes a codon specifying an amino acid to one specifying none, it is called a **nonsense mutation** (eg, a UAC [tyrosine] to UAA [STOP]), and the truncated product of the mutated gene is called a **nonsense fragment.** Microdeletions and microinsertions cause **frame shift mutations,** changes in the reading frame by which the ribosomes translate the mRNA from the mutated gene. Frame shifts usually result in polymerization of a stretch of incorrect amino acids until a nonsense codon is encountered, so the product is usually a truncated polypeptide fragment with an incorrect amino acid sequence at its N terminus. Deletion or insertion of a segment of base pairs from a gene shortens or lengthens the protein product if the number of base pairs deleted or inserted is divisible evenly by 3; otherwise it also brings about the consequence of a frame shift. Inversions of a small segment within a gene inactivate it; inverting larger segments may affect chiefly the genes at the points of inversion. Duplications, probably the most common of all mutations, serve an important role in the evolution of genes with new functions. Mutations are summarized in Table 4–1.

Changes in nucleotide sequence affect the synthesis of the protein products of genes

Many mutations, particularly if they occur near the end of a gene, prevent the expression of all genes downstream (away from the promoter) of the mutated gene. Such **polar mutations** are thought to exert their effect on neighboring genes by the termination of

Mutations may affect neighboring genes by termination of transcription

TABLE 4–1

**Mutations**

| Type | Causative Agent | Consequences |
|---|---|---|
| **Replacement** | | |
| Transition: pyrimidine replaced by a pyrimidine or a purine by a purine | Base analogs, ultraviolet radiation, deaminating and alkylating agents, spontaneous | Transitions and transversions: if nonsense codon formed, truncated peptide; if missense codon formed, altered protein |
| Transversion: purine replaced by a pyrimidine or vice versa | Spontaneous | |
| **Deletion** | | |
| Macrodeletion: large nucleotide segment deleted | $HNO_2$, radiation, bifunctional alkylating agents | Truncated peptide; other products possible, such as fusion peptides |
| Microdeletion: one or two nucleotides deleted | Same as macrodeletions | Frame shift, usually resulting in nonsense codon and truncated peptide |
| **Insertion** | | |
| Macroinsertion: large nucleotide segment inserted | Transposons or insertion sequence (IS) elements | Interrupted gene yielding truncated product |
| Microinsertion: one or two nucleotides inserted | Acridine | Frame shift, usually resulting in nonsense codon yielding a truncated product |
| **Inversion** | IS or IS-like elements | Many possible effects |

transcription of downstream genes when translation of the mRNA of the mutated gene is blocked by a nonsense codon.

Mutagens increase the natural frequency of mutation

There is a certain natural frequency of mutations brought about by errors in replication, but various environmental and biological agents can increase the frequency greatly. Different types of mutations are increased selectively by different agents, as listed in Table 4–1.

Common biological consequences of mutations include resistance to antimicrobics, nutritional requirements, and altered response to environment

Mutations in essential genes are not lethal if they are expressed only conditionally, as within a particular temperature range

Mutations may also be classified according to their biological consequences. Some mutations change the susceptibility of a cell to an antimicrobic or other toxic agent; these **resistance mutations** might, for example, affect the structure of certain cell proteins in such a way that the agent cannot enter the cell or cannot inactivate its normal target. Some mutations, called **auxotrophic mutations,** affect the production of a biosynthetic enzyme and result in a nutritional requirement of the mutant cell for the amino acid, nucleotide, vitamin, or other biosynthetic product it can no longer make for itself. The wild type from which the mutant was derived is said to be **prototrophic** for that nutrient. Some mutations affect a gene whose product is essential for growth and cannot be bypassed nutritionally; these are called **lethal mutations.** If the product of a mutated gene is active in some circumstances but inactive under others (eg, high or low temperature), the mutation is called **conditional** (meaning **conditionally expressed**). The most common kind of conditional mutation is one in which the protein product of the mutated gene is inactive at a normally physiologic temperature, but active at a higher or lower temperature; these are called **temperature-sensitive mutations.**

## Reversion and Suppression of Mutations

Back mutations are rare because highly specific corrections are needed

Suppressor mutations reestablish original phenotype

A **reversion,** or **back mutation,** is the conversion of a mutated gene back to its original wild-type allele. True back mutation can occur but at a low frequency, because a very specific and improbable event is required. Much more commonly observed is the conversion of a mutant cell into one that is phenotypically identical to the original wild-type bacterium for the affected character but still retains the original mutation. These **suppressor** mutations can arise in several ways. Within the mutated codon a second mutation can create a new codon specifying the original amino acid. Alternatively, secondary mutations in other codons of the mutated gene can lead to a change in amino acid sequence that results in an active product despite the continued presence of the original amino acid error. Suppressing mutations can occur even in genes other than the one that was originally mutated. For example, when two proteins interact to perform a function, the mutant form of one may be active when combined with a mutant form of the other. Another example involves tRNA molecules, the translators of the genetic code, which can themselves be altered by mutation; it is possible for a mutant tRNA to "mistake" a mutant codon and insert the original correct amino acid, a case of two wrongs making a right.

## Repair of DNA Damage

Several processes involving many genes operate to repair various sorts of damage to DNA

Many mutagenic agents directly alter the structure of DNA, and some are ubiquitous components of the environment (heat, sunlight, acid, oxidants, and alkylating agents). It is therefore not surprising to learn that bacteria have evolved multiple biochemical mechanisms for repairing damaged DNA. In *E. coli,* for example, more than 30 genes are known to be involved in DNA repair; many of these are members of the SOS response discussed in Chapter 3. Collectively these repair systems can remove thymine dimers produced by ultraviolet (UV) irradiation, can remove methyl or ethyl groups placed on guanine residues, can excise bases damaged by deamination or ring breakage and replace them with authentic residues, and can recognize and repair DNA depurinated by acid or heat. In large measure these repair systems use the fact that DNA is double stranded. Damage is recognized by the mispairing it causes, and the information on one strand is used to direct the proper repair of the damaged strand. Also, a proofreading process operates during DNA replication to detect any mismatch between each newly polymerized base and its mate in the template strand. Mismatches are excised to permit repolymerization with the properly matched nucleotide. Failures of this proofreading process can be detected and handled by an excision and resynthesis system similar to those that recognize and repair chemically damaged DNA.

One system bypasses DNA damaged by UV irradiation when repair has failed. It directs replication to proceed across a region badly damaged by the formation of thymine dimers. This **error-prone replication** is responsible for the mutations induced by UV light.

Some repair processes result in mutation

## GENETIC EXCHANGE

Mutation and selection are important factors in bacterial evolution, but evolution proceeds far faster than it could by these processes alone. For instance, the probability that the process of random mutation alone can produce a cell that, let us say, requires five mutations for optimal growth in a new environment is extremely low. It is in fact the product of the individual mutation frequencies (eg, $10^{-6} \times 10^{-6} \times 10^{-6} \times 10^{-6} \times 10^{-6} = 10^{-30}$), and that essentially precludes a natural population from ever acquiring the new property in this manner. However, such alterations occur because organisms exchange genetic material, thereby permitting combinations of mutations to be collected in individual cells.

Evolution is speeded by exchange of genetic material

Despite the fact that bacteria reproduce exclusively asexually, the sharing of genetic information within and between related species is now recognized to be quite common and to occur in at least three fundamentally different ways. All three processes involve a one-way transfer of DNA from a **donor cell** to a **recipient cell.** The molecule of DNA introduced into the recipient is called the **exogenote** to distinguish it from the cell's own original chromosome, called the **endogenote.**

One-way passage of DNA from a donor to a recipient adds an exogenote to the recipient endogenote

One process of DNA transfer, called **transformation,** involves the release of DNA into the environment by the lysis of some cells, followed by the direct uptake of that DNA by the recipient cells. By another means of transfer, called **transduction,** the DNA is introduced into the recipient cell by a nonlethal virus that has grown on the donor cell. The third process, called **conjugation,** involves actual contact between donor and recipient cell during which DNA is transferred as part of a plasmid (an autonomously replicating, extrachromosomal molecule of circular double-stranded DNA); in conjugation, donor and recipient cells are referred to as male and female, respectively. The three means of gene transfer are summarized in Figure 4–2.

Transformation, transduction, and conjugation are the major processes of DNA transfer

Species of bacteria differ in their ability to transfer DNA, but all three mechanisms are distributed among both Gram-positive and Gram-negative species; however, only transformation is governed by bacterial chromosomal genes. Transduction is totally mediated by virus genes, and conjugation, by plasmid genes.

Transformation, transduction, and conjugation are mediated by chromosomal, viral, and plasmid genes, respectively

### Transformation

Transformation was first demonstrated in 1928 by F. Griffith, a British public health officer, who showed that virulent, encapsulated *Streptococcus pneumoniae* (pneumococci) that had been killed by heat could confer on living, avirulent, nonencapsulated pneumococci the ability to make the polysaccharide capsule of the killed organisms and thus become virulent for mice. Subsequent work in 1944 by O. T. Avery, C. M. MacLeod, and M. McCarty at the Rockefeller Institute revealed that the "transforming factor" from the dead pneumococci was nothing other than DNA. This discovery had enormous impact on biology, because it was the first rigorous demonstration that DNA is the macromolecule in which genetic information is encoded. It opened the door to modern molecular genetics.

Studies on pneumococcal transformation led to identification of genetic material

The ability to take up DNA from the environment is called **competence,** and in many species of bacteria, it is encoded by chromosomal genes that become active under certain environmental conditions. In such species, transformation can occur readily and is said to be natural. Other species cannot enter the competent state but can be made permeable to DNA by treatment with agents that damage the cell envelope making an **artificial transformation** possible.

Genes encoding competence enable uptake of DNA; species lacking them must be made permeable

Natural transformation must be important in nature, judged by the variety of mechanisms that different bacteria have evolved to accomplish it. Two of the best-studied systems are those of the Gram-positive pneumococcus and a Gram-negative rod, *Haemophilus influenzae*. Pneumococcal cells secrete a protein **competence factor** that induces many of the cells of a culture to synthesize special proteins necessary for transformation, including an autolysin that exposes a cell membrane DNA-binding protein. Any DNA present in the medium is bound indiscriminately; even salmon sperm DNA can be bound and taken up as

Pneumoccal competence involves a nonspecific DNA-binding protein

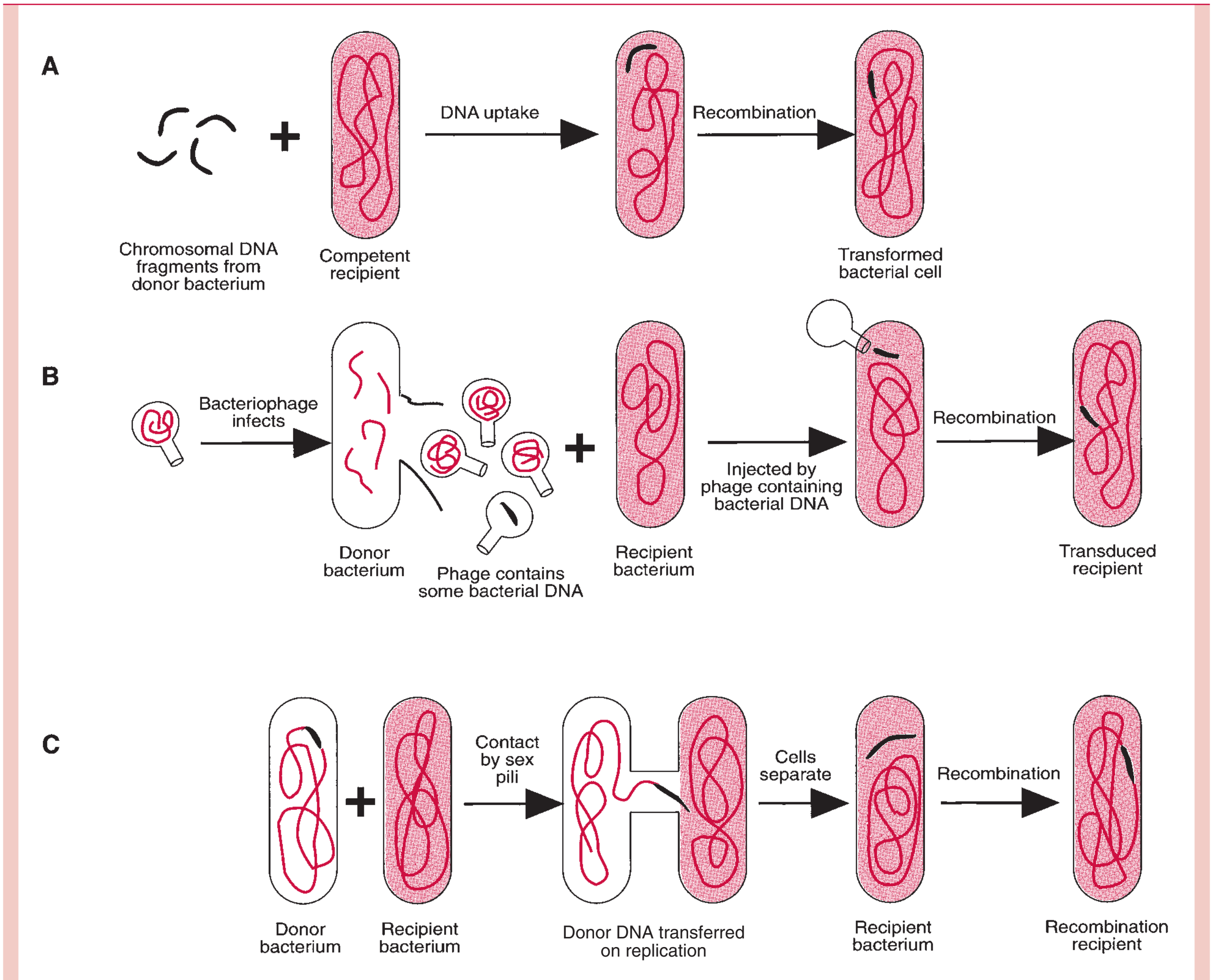


FIGURE 4–2
Chromosomal gene transfer mechanisms in bacteria. **A.** Transformation. **B.** Transduction. **C.** Conjugation.

readily as DNA from another pneumococcal cell. The surface-bound double-stranded DNA is cleaved into fragments of about 6 to 8 kilobases (kb). One strand is degraded by a nuclease, while the complementary strand of each fragment is taken up by a process that seems to be driven by the proton-motive force of the cell membrane (see Chapter 3). The fate of the internalized DNA fragment then depends on whether it shares homology (the same or similar in base sequence) with a portion of the recipient cell's DNA. If so, recombination can occur by a process described later, but heterologous DNA (no similarity to the endogenote) is degraded and causes no heritable change in the recipient.

All DNA is taken up, but heterologous DNA is degraded

Transformation in *H. influenzae* is somewhat different. There is no competence factor, and cells become competent merely by growth in an environment rich in nutrients. Only homologous DNA (ie, DNA from the same or a closely related species of *Haemophilus*) is taken up, and it is taken up in double-stranded form. The selectivity is brought about by the presence of a special membrane protein that binds to an 11-base pair (bp) sequence (5'-AAGTGCGGTCA-3') that occurs frequently in *Haemophilus* DNA and infrequently in other DNAs. Following binding to molecules of this protein, the homologous DNA is internalized by a mechanism that resembles membrane invagination, resulting in the temporary residence of the exogenote in cytosolic membrane vesicles. Although the DNA taken up is double stranded, only one of the two strands participates in the subsequent recombination with the endogenote.

*H. influenzae* endocytoses only homologous dsDNA, recognized by a characteristic 11-bp sequence

The common use of *E. coli* as a host cell in which to clone genes on hybrid plasmids (see Invertible DNA Segments and Recombinational Regulation of Gene Expression) depends on procedures involving treatment with salt and temperature shocks to bring about artificial transformation; this organism has no natural competence mechanism. In contrast, the pathogen, *Neisseria gonorrhoeae* regularly uses transformation to bring about changes in the antigenic nature of its pili, as described later in the section on recombination.

Transformation is common among many pathogens; artificial transformation enables use of *E. coli* for gene cloning

## Transduction

Transduction is virus-mediated transfer of genetic information from donor to recipient cell. To understand transduction and its several mechanisms, it is necessary to preview the nature of bacterial viruses, a topic dealt with more extensively in Chapters 5, 6, and 7.

Viruses are capable of reproduction only inside living cells. Those that grow in bacteria are called **bacteriophages**, or simply **phages.** They are minimally composed of protein and nucleic acid, although some may have a very complex structure and composition. The individual virus particle or virion consists of a protein capsid enclosing genomic nucleic acid, which is either RNA or DNA, but never both. Virions infect sensitive cells by adsorbing to specific receptors on the cell surface and then, in the case of phages, injecting their DNA or RNA. Phages come in two functional varieties according to what happens after injection of the viral nucleic acid. **Virulent** (lytic) **phages** cause lysis of the host bacterium as a culmination of the synthesis of many new virions within the infected cell. **Temperate phages** may initiate a lytic growth process of this sort or can enter a quiescent form (called a **prophage**), in which the infected host cell is permitted to proceed about its business of growth and division but passes on to its descendants a prophage genome capable of being **induced** to produce phage in a process nearly identical to the growth of lytic phages. The bacterial cell that harbors a latent prophage is said to be a **lysogen** (capable of producing lytic phages), and its condition is referred to as **lysogeny**. Lysogens are immune to infection by virions of the type they harbor as prophage. Occasionally, lysogens are spontaneously induced and lysed by the phage and release mature virions (as many as 75 to 150 or more per cell) into the environment. When triggered by UV irradiation or certain chemicals, an entire population of lysogens are induced simultaneously to initiate reproduction of their latent virus followed by lysis of the host cells. Infection of a sensitive cell with the temperate phage can lead to either lysis or lysogeny. How this choice comes about is described in Chapter 7.

Phages are viruses of diverse structure and modes of replication

Virulent phages produce new virions in the host bacterial cell, usually lysing it

Temperate phages can either lyse a bacterial host cell or lysogenize it as a prophage

Prophage induction leads to virion production and cell lysis

The prophage of different temperate phages exists in one of two different states. In the first, the prophage DNA is physically integrated into a bacterial chromosome; in the second, it remains separate from the chromosome as an independently replicating, circularized, molecule of DNA. Prophages of this sort are in fact plasmids.

Some prophages integrate; others behave as plasmids

For the most part, transduction is mediated by temperate phage, and the two broad types of transduction result from the different physical forms of prophage and the different means by which the transducing virion is formed. These are termed **generalized transduction,** by which any bacterial gene stands an equal chance of being transduced to a recipient cell, and **specialized** or **restricted transduction,** by which only a few genes can be transduced.

Transduction, whether generalized or specialized, is mediated by temperate phage

### *Generalized Transduction*

Some phages package DNA into their capsids in a nonspecific way, the headful mechanism, in which any DNA can be stuffed into the capsid head until it is full. (The head is the principal structure of the virion to which, in some cases, a tail is attached; see Chapter 5.) An endonuclease then trims off any projecting excess. If fragments of host cell DNA are around during the assembly of mature virions, they can become packaged in place of virus DNA, resulting in **pseudovirions.** Pseudovirions are the transducing agents. They can adsorb to sensitive cells and inject the DNA they contain as though it were viral DNA. The result is the introduction of donor DNA into the recipient cell.

In generalized transduction, pseudovirions inject a random piece of host DNA into a recipient

Any given gene has an equal probability of being transduced by this process. With the temperate phage P1 of *E. coli,* this probability is approximately one transduction event per $10^5$ to $10^8$ virions, because nearly 1 out of every 1000 phage particles made in a P1

Genes have low but equal probability of being transduced lytic infection are pseudovirions, and the bacterial DNA fragments packaged are 1 to 2% of the length of the chromosome. Cotransduction of two bacterial genes by a single pseudovirion occurs only if they are located close together within this small length of the chromosome, and this fact facilitates mapping the position of a newly discovered gene.

Once injected into the host cell, the transduced DNA is lost by degradation unless it can recombine with the chromosome of the recipient cell, usually by homologous recombination (see below, Invertible DNA Segments and Recombinational Regulation of Gene Expression) in which both strands of the exogenote cross into and replace the homologous segment of the recipient's chromosome. However, sometimes the exogenote can persist without degradation by assuming a stable circular configuration.

### *Specialized Transduction*

Specialized transduction involves imprecise excision of an integrated prophage

A few genes adjacent to the prophage are transferred to the recipient and cointegrated with prophage

It has been noted that the prophage of some phages is integrated into the lysogen's chromosome. This integration does not occur haphazardly but is restricted to usually one site, called the *att* (attachment) site. When a lysogen carrying such a prophage is induced to produce virions, excision of the viral genome from the bacterial chromosome occasionally (eg, in 1 of $10^5$ to $10^6$ lysogens) occurs imprecisely, resulting in a pickup of genes of the bacterium adjacent to the *att* site. The resulting virion may be infectious (if no essential phage genes are missing) or defective (if one or more essential genes are missing). In either case, adsorption to a sensitive cell and injection of the DNA can occur, and integration of the aberrant phage genome into the chromosome of the new host cell results in the formation of a lysogen containing a few genes that have been transduced as hitchhikers with the phage genome. Integration of the phage genome automatically accomplishes the recombinational event needed to guarantee reproduction of the transduced genes. Only genes that border the *att* site stand a chance of being transduced by this process, which is why it is called specialized or restricted transduction.

All virions produced by lysogenic transductants carry original transduced gene

Because the original pickup event is rare, the first transducing process is termed **low-frequency transduction;** however, when a lysogenic transductant is, in turn, induced to produce phage, all of the new virions carry the originally transduced bacterial gene. The resulting mixture of lysed cells and virions now brings about **high-frequency transduction** of the attached genes.

Specialized transduction has been valuable in gene cloning and sequencing

Bacterial geneticists have learned to move genes of interest near the phage integration site and thereby construct specialized transducing phages containing these genes. Such transducing phages are valuable aids to cloning and sequencing genes and to studying their function and regulation. Obviously a temperate phage that could form a prophage by integrating randomly at any site in the bacterial chromosome would be of special use. The temperate phage Mu of *E. coli* has this property.

Transduction is common in nature, important clinically, and useful in research

Although both generalized transduction and specialized transduction can be regarded as the result of errors in phage production, transfer of genes between bacterial cells by phage is a reasonably common phenomenon. It occurs at significant frequency in nature; for example, genes conferring antimicrobic resistance in staphylococci are often transduced from strain to strain in this way. The toxins responsible for the severe clinical symptoms of diphtheria and of cholera are encoded by genes transduced into *Corynebacterium diphtheriae* and *Vibrio cholerae,* respectively. Transduction is also used extensively as a tool in molecular biology research.

## Conjugation

Conjugation is plasmid-encoded and requires cell contact

Conjugation is the transfer of genetic information from donor to recipient bacterial cell in a process that requires intimate cell contact; it has been likened to mating. By themselves, bacteria cannot conjugate. Only when a bacterial cell contains a self-transmissible **plasmid** (see below for definition) does DNA transfer occurs. In most cases, conjugation involves transfer only of plasmid DNA; transfer of chromosomal DNA is a rarer event, and is mediated by only a few plasmids. Plasmids are of enormous importance to medical microbiology. They are discussed in detail later in this chapter, but to understand conjugation we should introduce some of their features at this point.

**Plasmids** are autonomous extrachromosomal elements composed of circular double-stranded DNA; a few rare linear examples have been found. Plasmids are found in most species of Gram-positive and Gram-negative bacteria in most environments. Plasmids govern their own replication by means of special sequences and proteins. They replicate within the host cell (and only within the host cell) and are partitioned between the daughter cells at the time of cell division. In addition, many plasmids are able to bring about their own transfer from one cell to another by the products of a group of genes called ***tra*** (for transfer); such plasmids are called **conjugative plasmids.** Other plasmids, called **nonconjugative,** lack this ability. The *tra* genes, of which there may be dozens, encode the structures and enzymes that accomplish conjugation. One of these structures is a specialized pilus (see Chapter 2) called the **sex pilus,** which confers the ability to seize recipient cells on the plasmid-containing donor cells. Retraction of the pilus draws the donor and recipient cell into the intimate contact needed to form a conjugal bridge through which DNA can pass. One strand of the plasmid DNA is then enzymatically cleaved at a site called the **origin of transfer** (***oriT***), and the resulting 5' end of the strand is guided into the recipient cell by the action of various *tra*-encoded proteins (Fig 4–3). Both the introduced strand and the strand remaining behind in the donor cell direct the synthesis of their complementary strand in a process called **transfer replication,** resulting in complete copies in both donor and recipient cell. Finally, circularization of the double-stranded molecules occurs, the conjugation bridge is broken, and both cells can now function as donor cells.

Plasmids are ubiquitous in most bacterial species

Conjugative plasmids can transfer themselves through activity of *tra* genes

Transfer replication ensures retention of plasmid copy in donor

Conjugation is a highly evolved and efficient process. Suitable mixtures of donor and recipient cells can lead to nearly complete conversion of all the recipients into donor, plasmid-containing cells. Furthermore, although some conjugative plasmids can transfer themselves only between cells of the same or closely related species, others are quite promiscuous, promoting conjugation across a wide variety of (usually Gram-negative) species. Conjugation appears to be a carefully regulated process, normally kept in check by the production of a repressor encoded by one of the *tra* genes. Interestingly, nonconjugative plasmids that happen to inhabit a cell with a conjugative plasmid can under some circumstances be transferred due to the conjugation apparatus of the latter; this process is called **plasmid mobilization.** As the later discussion of plasmids shows, their conjugal properties have enormous implications in medicine.

Conjugation is efficient, well regulated, and may cross species lines

Nonconjugative plasmids can be transferred by plasmid mobilization

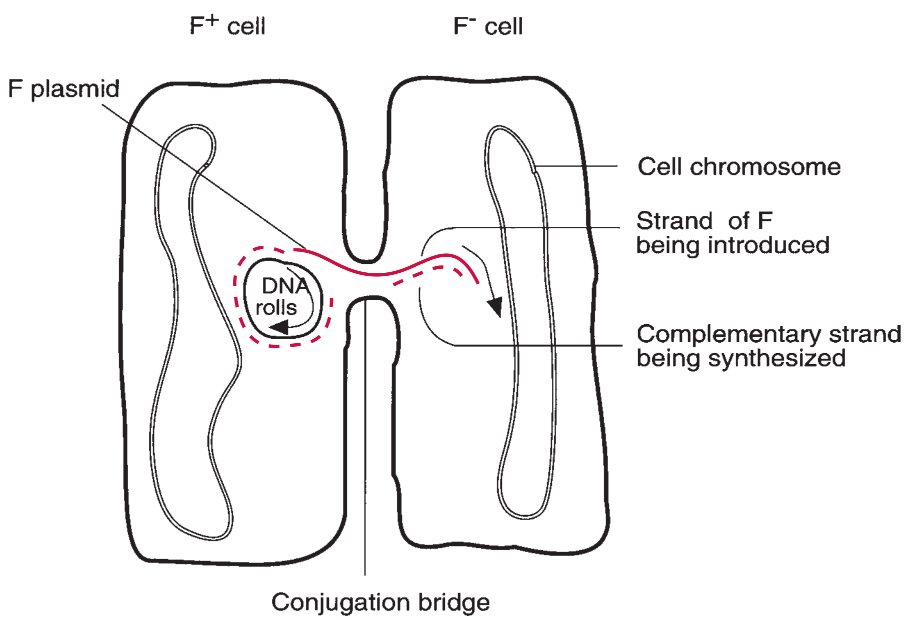


FIGURE 4–3
Bacterial conjugation resulting in the introduction of an F plasmid into an $F^-$ cell by replicative transfer from an $F^+$ cell.

## *Conjugation in Gram-Negative Species*

F factor is a conjugative plasmid that can transfer bacterial chromosome genes

After many inconclusive attempts by microbiologists to learn whether a sexual process of genetic exchange existed among bacteria, J. Lederberg and E. Tatum discovered conjugation in 1946. What they observed was a transfer of chromosomal genes between cells of two different strains of *E. coli*. Their discovery stimulated an intensive analysis of the mechanism, leading to the discovery of an agent, the **F factor** (for fertility factor), that conferred on cells the ability to transfer bacterial chromosome genes to recipient cells. Now it is recognized that the F factor is a conjugative plasmid, although an atypical one in several respects.

The F plasmid is a normal conjugative plasmid in that it possesses many *tra* genes encoding a sex pilus (the **F-pilus**) as well as the ability to form a conjugation bridge, to initiate transfer replication, and to perform all the other steps of plasmid transfer. Thus, a cell harboring the F plasmid (an **F$^+$ cell**) can conjugate with a recipient **F$^-$ cell,** and in the process the latter becomes F$^+$. The process is immediate and efficient because the F factor has lost autoregulation of the conjugation process. However, these properties do not explain how the F plasmid can bring about transfer of chromosomal genes, which is more closely related to another property of F—its ability to integrate at low frequency into the bacterial chromosome at seven or eight chromosomal sites, resulting in linearization of the plasmid DNA as part of the giant circular chromosomal molecule. A cell in which this integration event has occurred is designated a **high-frequency recombination** (Hfr) cell; it is only this spontaneous mutant in an F$^-$ population that transfers donor chromosomal genes. When an Hfr cell encounters an F$^-$ cell, conjugation occurs and the usual transfer replication is initiated at *oriT*, within the linear F segment. However, in this circumstance, breaking the integrated plasmid DNA at *oriT* results in the formation of a linear strand in which the entire bacterial chromosome lies between two portions of the F genome (Fig 4–4), and therefore the leading segment of F enters the F$^-$ cell followed by bacterial genes one after the other. The conjugation bridge usually ruptures long before the entire bacterial chromosome can be introduced, resulting in the transfer of only one part of the F genome and a variable length of the bacterial chromosome. Thus, conjugation between an Hfr and an F$^-$ cell leaves the recipient still F$^-$, but having received bacterial genes; the donor remains Hfr because it retains a copy of the chromosome with its integrated F genome. There are other fertility plasmids, but F remains the best studied.

Rare integration of F into the bacterial chromosome leads to transfer of chromosomal genes during conjugation

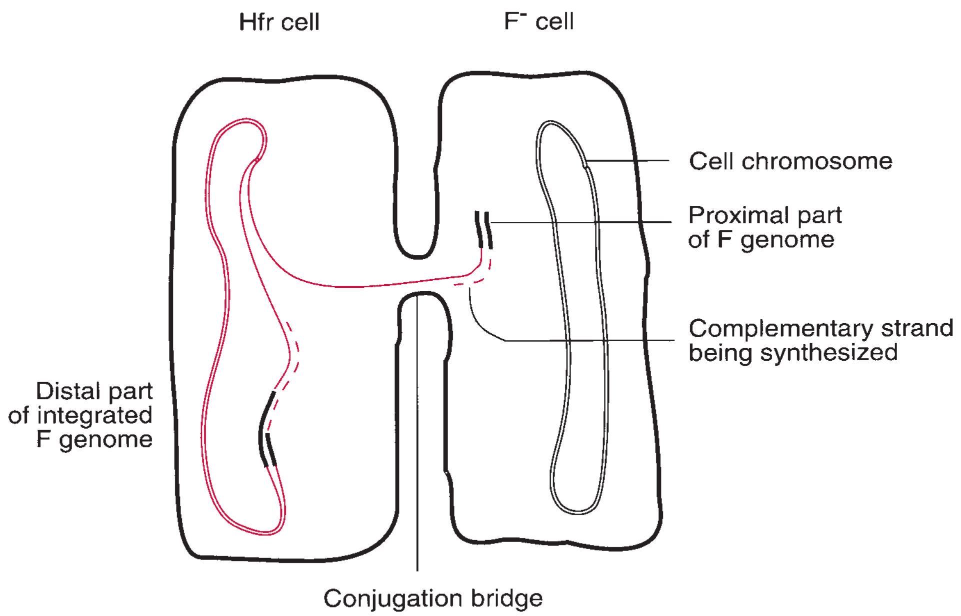


FIGURE 4–4

Bacterial conjugation resulting in the introduction of chromosomal genes and a portion of the F plasmid genome into an F$^-$ cell by replicative transfer from a high-frequency recombination (Hfr) cell.

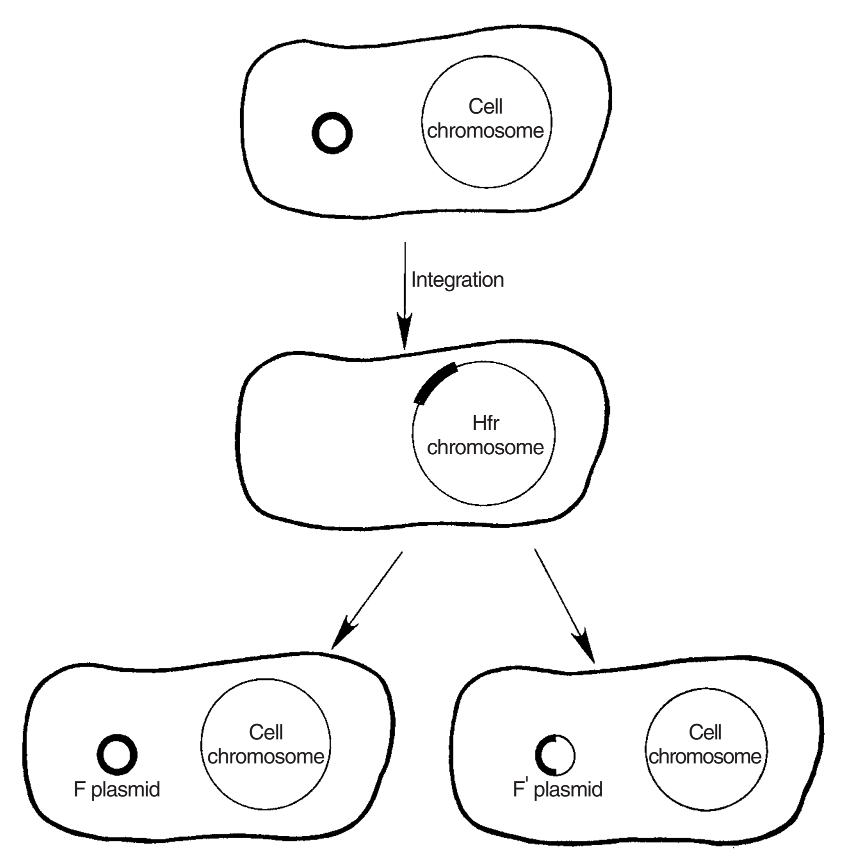


FIGURE 4–5
Integration of the F plasmid into a bacterial chromosome to form a high-frequency recombination (Hfr) chromosome, followed either by exact excision to reform the F plasmid or by inexact excision to form an F′ plasmid containing some bacterial chromosome genes.

There is an additional wrinkle to the transfer of chromosomal genes by conjugation in *E. coli*. It is a process termed **sexduction,** in which an F plasmid transfers from one cell to another a few bacterial chromosomal genes that it happens to contain. This comes about because the F genome in an Hfr cell can, at low frequency, excise itself from the chromosome and circularize into plasmid form. When this excision is imperfect, or involves recombinations with other insertion sites, segments of the bacterial chromosome can become included in the plasmid (Fig 4–5). When the resulting plasmid, called F′ to note its content of some bacterial DNA, is transmitted to recipient cells at high frequency by conjugation, the chromosomal genes are transferred as hitchhikers; this is the process of sexduction. By similar processes, segments of bacterial chromosomes can become incorporated into other plasmids, discussed later in this chapter, that confer resistance to antimicrobics.

Hybrid F′ plasmids can include segments of the bacterial chromosome and transfer them at high frequency to $F^-$ cells during conjugation

## *Conjugation in Gram-Positive Species*

Plasmids carrying genes encoding antimicrobic resistance, common pili and other adhesins, and some exotoxins are readily transferred by conjugation among Gram-positive bacteria in the natural environment as well as in the laboratory. However, conjugation involving chromosomal genes may differ between Gram-negative and Gram-positive species, as judged by its characteristics in two well-studied examples, *E. coli* and *Enterococcus faecalis.* Conjugation in *E. faecalis* is mediated by plasmids, but there is also an involvement of chromosomal genes in the process. Donor and recipient cells do not couple by means of a sex pilus but rather by the clumping of cells that contain a plasmid with those that do not. This clumping is the result of interaction between a proteinaceous **adhesin** on the surface of the donor (plasmid-containing) cell and a **receptor** on the surface of the recipient (plasmid-lacking) cell. Both types of cells make the receptor (possibly cell wall lipoteichoic acid), but only the plasmid-containing cell can make the adhesin, presumably because it is encoded by a plasmid gene. Interestingly, donor cells make the adhesin only when in the vicinity of recipient

*E. faecalis* coupling results from adhesin–receptor interaction

Plasmid-encoded *E. faecalis* adhesin is produced in response to recipient pheromone

cells because the recipients secrete small peptide **pheromones** that serve to notify the donor cells of the presence of recipients. Donor cells promptly make adhesin when they sense the pheromone. As a result, clumps are formed, and plasmid DNA is transferred across conjugation bridges into the recipient cells held in the clumps.

Some Gram-positive conjugal transfers may be mediated by DNA elements that are only transiently plasmids

In addition to enterococcal species, species of *Bacillus*, *Staphylococcus,* and *Clostridium* have been found to contain conjugative plasmids. Conjugative transfer of genes has also been observed in a number of Gram-positive species in the apparent absence of plasmid DNA. In several instances these transfers involve conjugative transposons (to be discussed later in this chapter), and it appears that a plasmid intermediate is formed, although only transiently.

Before continuing with our discussion of plasmids, we should complete the story of what happens to DNA introduced into recipient cells by any of the three transfer processes, transformation, transduction, and conjugation.

## GENETIC RECOMBINATION

Exogenote may be degraded, circularized, or integrated into recipient chromosome

By whatever means an exogenote is conveyed into a recipient cell, its effect depends on what happens after transfer. There are basically three possible fates. The exogenote DNA may be degraded by a nuclease, in which case no heritable change is brought about. It may be stabilized by circularization and remain separate from the endogenote. In this case, if it is unable to replicate, it will be unilinearly inherited (eg, abortive transduction). If it is capable of self-replication, it will become established as an autonomous, inherited plasmid. The third possible fate is **recombination** between exogenote and endogenote, resulting in the formation of a partially hybrid chromosome with segments derived from each source. These possibilities are diagrammed in Figure 4–6.

In this section we examine the two principal processes by which recombinant chromosomes are formed following genetic transfer: homologous recombination and site-specific recombination. A third sort of recombinational process exists, called **illegitimate recombination,** because it does not obey the legitimate laws governing homologous

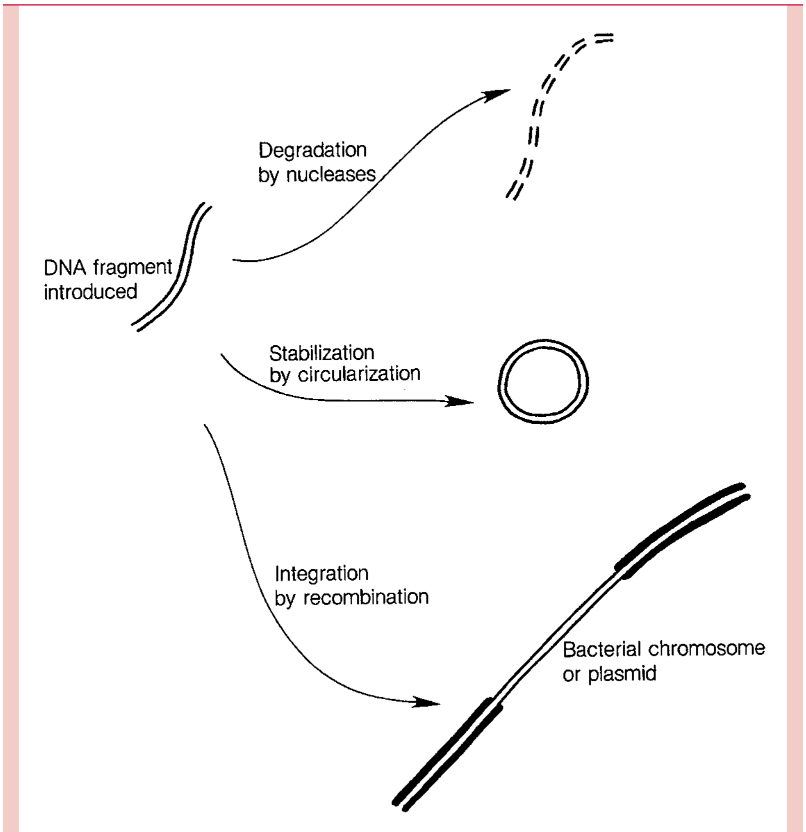


FIGURE 4–6
Possible fates of a DNA fragment after transfer into a bacterial cell.

and site-specific recombination. Little is known other than it results in some types of gene duplications and deletions, and this chapter shall say no more about it.

## Homologous Recombination

One mechanism by which an exogenote can recombine with the bacterial chromosome is called **homologous recombination.** This term reflects one of the two requirements for this process: (1) the exogenote must possess reasonably large regions of nucleotide sequence identity or similarity to segments of the endogenote chromosome, because extensive base pairing must occur between strands of the two recombining molecules; and (2) the recipient cell must possess the genetic ability to make a set of enzymes that can bring about the covalent substitution of a segment of the exogenote for the homologous region of the endogenote. Not all the details are known, but the latter process includes breaking one strand of each recombining molecule at a time and pairing it with the unbroken, complementary strand of the other molecule. The ends of the broken strands are partially digested, then repaired and joined so that the rejoined strands are now continuous between the chromosomes. A protein known as RecA (recombination) controls the entire process. The same **breakage** and **reunion** process then links the second strand of each recombining DNA molecule. This **crossover** event repeated further down the chromosome results in the substitution of the exogenote segment between the two crossovers for the homologous segment of the endogenote. This process is schematically presented in a very simplified form in Figure 4–7. Homologous recombination is responsible for integration of DNA fragments transferred by generalized transduction, by plasmid-mediated conjugation, and by natural transformation.

Homologous recombination involves nucleotide similarity and specific enzymes such as RecA

Homologous recombination can follow generalized transduction, conjugation, or transformation

## Site-Specific Recombination

The second major type of recombination is actually a group of separate mechanisms that are RecA independent, that rely on only limited DNA sequence similarity at the sites of crossover, and that are mediated by different sets of specialized enzymes designed to catalyze recombination of only certain DNA molecules. The name for this large group of mechanisms, **site-specific recombination,** reflects the fact that these recombinational events are restricted to specific sites on one or both of the recombining DNA molecules. The enzymes that bring about site-specific recombination operate not on the basis of

Site-specific recombination is RecA independent and requires enzymes that operate only on unique sequences

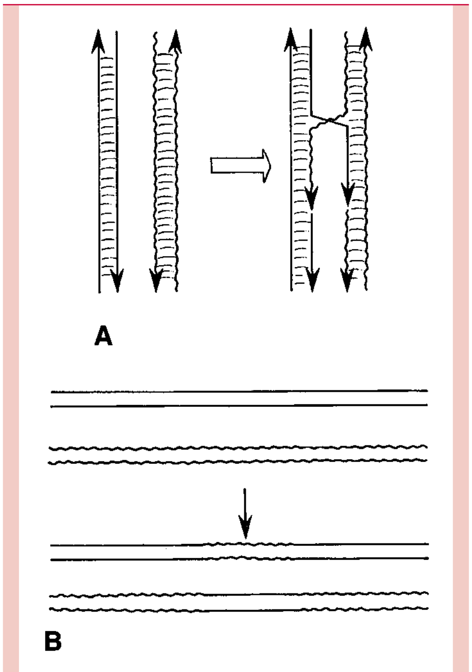


FIGURE 4–7
Homologous recombination. **A.** Central event in homologous recombination. Extensive base pairing between homologous regions of strands of two DNA molecules is illustrated. Events that accompany or follow this event include strand nicking, migration of the crossover point with partial digestion of the nicked strands, and resynthesis and ligation. Both strands of both recombining molecules must participate to effect a crossover event. **B.** Result of homologous recombination. Two crossover events are necessary to achieve the exchange of segments shown.

Enzymes are usually encoded by exogenote genes

DNA homology but on recognition of unique DNA sequences that form the borders of the specific sites. These enzymes are commonly encoded by genes on the exogenote.

Integration of many prophages occurs by site-specific recombination

One good example of site-specific recombination has already been shown. The integration of some phage genomes into the chromosome occurs only at one site on the bacterial chromosome and one site on the phage chromosome. It was noted briefly that some phages, notably phage Mu, differ in being able to integrate almost anywhere in the bacterial chromosome. Because the site of recombination (the crossover site) in the Mu genome is the same in all cases, this, too, is a case of site-specific recombination.

In addition to the special kind of recombination represented by prophage integration, a particular form of site-specific recombination occurs in other situations of enormous consequence to medical microbiology. These involve special genetic units called transposable elements, which have proven to be so important in the life of bacteria, particularly in their roles in the pathogenesis of infectious disease, that a separate section must be devoted to their description.

## TRANSPOSABLE ELEMENTS

Transposable elements are genetic units that move within and between chromosomes and plasmids by means of specific transposases

**Transposable elements** are genetic units that are capable of mediating their own transfer from one chromosome to another, from one location to another on the same chromosome, or between chromosome and plasmid. This **transposition** relies on their ability to synthesize their own site-specific recombination enzymes, called **transposases.**

The three major kinds of transposable elements are **insertion sequence** elements; **transposons;** and certain prophages, such as Mu.

### Insertion Sequence Elements

IS elements encode only proteins for their own transposition

**Insertion sequence (IS) elements** are segments of DNA of approximately 1000 bp. They encode enzymes for site-specific recombination and have distinctive nucleotide sequences at their termini. Different IS elements have different termini, but, as illustrated in Figure 4–8, a given IS element has the same sequence of nucleotides at each end, but in an inverted order. Only genes involved in transposition (eg, one encoding a transposase) and in the regulation of its frequency are included in IS elements, and they are therefore the simplest transposable elements.

Insertion of IS elements into a gene causes mutation

Because IS elements contain only genes for transposition, their presence in a chromosome is not always easy to detect. However, if an IS element transposes to a new site that is within a gene, this insertion is actually a mutation that alters or destroys the activity of the gene. Because most IS elements contain a transcription termination signal, the insertion also eliminates transcription of any genes downstream in the same operon. This property of IS elements led to their first recognition. Reversion of insertion mutations can occur by deletion, but the frequency of deletion is 100- to 1000-fold lower than that of insertion.

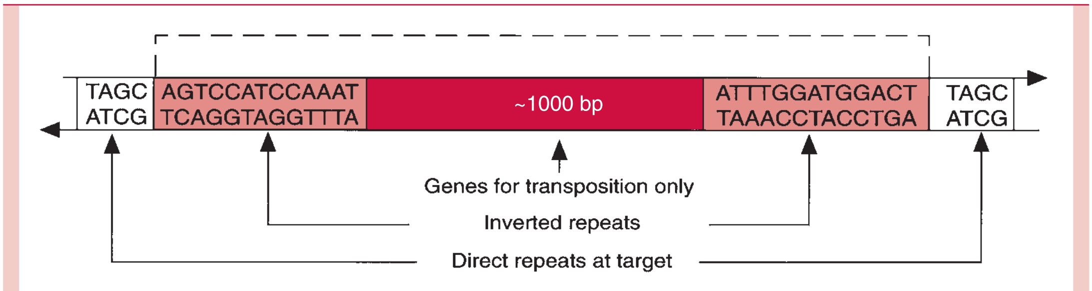


FIGURE 4–8
Structure of an insertion sequence (IS) element. The general features of bacterial IS elements are illustrated. As an example, IS2 has a total of 1327 bp, of which there are terminal inverted repeat sequences of 41 bp flanking the central region that encodes the one or two proteins required for transposition of IS2. A direct repeat of 5 bp was created at the site of insertion of the element. Approximately five IS2 elements are found in the chromosome of many strains of *Escherichia coli.*

Numerous IS elements reside naturally at different locations in *E. coli* chromosomes and in *E. coli* plasmids, and this has many consequences for the cell. Because their size is sufficient to permit strong base pairing between different copies of the same IS element, they can provide the basis for RecA-mediated homologous recombination. In this manner, the presence of particular IS elements in both the F plasmid and the bacterial chromosome provides a means for the formation of Hfr molecules by cointegration using IS sequence homology and the RecA system.

Base pairing between copies of IS elements can promote homologous recombination

## Transposons

One of the major aspects of IS elements is that they are components of **transposons** (Tn elements), which are transposable segments of DNA containing genes beyond those needed for transposition. Transposons are as much as 10-fold larger than IS elements. One class, of which transposon Tn10 is a good example, are composite structures consisting of a central area of genes bordered by IS elements. The genes may code for such properties as antimicrobic resistance, substrate metabolism, or other functions. A generalized transposon structure of the Tn10 variety is shown in Figure 4–9.

Transposons encode functions beyond those needed for their own transposition

Some transposons are bordered by IS elements

Composite transposons of the Tn10 sort can translocate by what is called simple or **direct transposition,** in which the transposon is excised from its original location and inserted without replication into its new site. A second class, typified by transposon Tn3, has inverted repeat sequences rather than IS elements at its ends and encodes not only a transposase but also an enzyme called a **resolvase.** Transposition of Tn3 involves formation of a **cointegrate** of the two DNA molecules (or segments of the same molecule) involved in the transposition—that is, the one carrying the Tn3 and the one serving as the target. Replication of the transposon then occurs, and the resolvase separates (resolves) the cointegrate, restoring the two DNA molecules, each now with its own copy of Tn3. Transposition of this sort is called **replicative** or **duplicative transposition.**

Direct transposition moves the transposon from its original site to a new site

Replicative transposition leaves a copy of the transposon at its original site

Besides the primary insertion reaction, all transposable units promote other types of DNA rearrangements, including deletion of sequences adjacent to a transposon, inversion of DNA segments, fusion of separate plasmids within a cell, similar fusions that integrate plasmids with the cell chromosome, and repeated duplications that result in **amplification** of genes within transposons. All of these events have great significance for understanding the formation and spread of antimicrobic resistance through natural populations of pathogenic organisms. These subjects are discussed in the description of plasmids in the next section.

Transposons promote many changes in DNA

Some strains of streptococci harbor transposon-like, drug-resistance elements within their chromosome that are capable of mediating their own transfer to other cells by conjugation. One such **conjugative transposon** is Tn916, found originally in a strain of *E. faecalis*. This element, approximately 16 kb in size, contains a gene for tetracycline resistance. It and similar elements resemble transposons in many respects, including size, multiple target sites, ability to transfer from a chromosome to a plasmid, and ability to be removed from a plasmid or a chromosome by precise excision. What is unusual, however, is their ability to mediate their own intercellular transfer. It now appears that Tn916, and presumably similar elements, can form a transient plasmid-like structure as part of the process of conjugational transfer.

Conjugative transposons can mediate their own transfer between cells

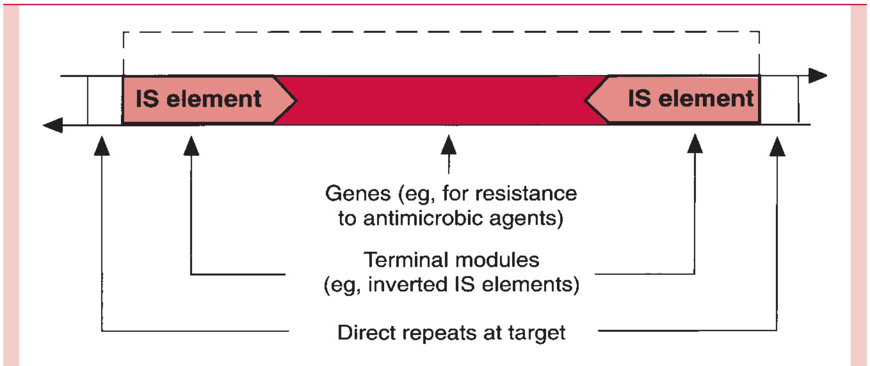


FIGURE 4–9
Structure of a composite transposon. The general features of bacterial transposons resembling Tn10 are illustrated. Tn10 has a total of 9500 bp. It consists of terminal direct-repeat IS10 elements flanking a central region that contains a gene for tetracycline resistance and genes needed for transposition.

The prophage of phage Mu is a transposon

The third type of transposable element is **transposable prophage,** such as that of bacteriophage Mu, which has the alternative of lytic growth or of lysogeny. During lysogeny, the prophage of Mu can insert virtually anywhere in the *E. coli* chromosome and later can transpose itself from one location to another. In fact, it is a transposon. When it integrates within a bacterial gene, it inactivates it in the same manner as any other transposable element. It was originally recognized as a virus that causes mutation, hence its name.

## Invertible DNA Segments and Recombinational Regulation of Gene Expression

Phase variation can be brought about by a recombinational event

A fascinating aspect of DNA rearrangements brought about by genetic recombination is that the expression of some chromosomal genes important in virulence are actually controlled by recombinational events. All the known cases involve **phase variation** of surface antigens. In *N. gonorrhoeae,* the bacteria that causes gonorrhea (see Chapter 20), multiple genes encoding antigenically different pilin sequences exist throughout the chromosome. Many, called *pilS*, are silent because they lack effective promoters; some are only fragments of pilin sequences. These silent genes or gene fragments serve as a reservoir of antigenic variability; each can, wholly or in part, become inserted by RecA-dependent homologous recombination into an actively expressed gene (*pilE*), resulting in the synthesis of a new pilin. The entire process resembles the insertion of cassette tapes into a tape player and, therefore, is referred to as the **cassette mode** of gene regulation (Fig 4–10).

Invertible elements can act as a genetic switch

A different DNA rearrangement is responsible for the alternation of expression of antigenically distinct flagellins, H1 and H2, in *Salmonella* species. An **invertible element** of 995 bp lies between the two flagellin genes (Fig 4–11). The phase-2 encoding gene (B) lies in an operon that also encodes a repressor for the phase-1 encoding gene (C). The latter gene is, therefore, active only if the former operon is inactive. Activity of the phase-2 operon, which

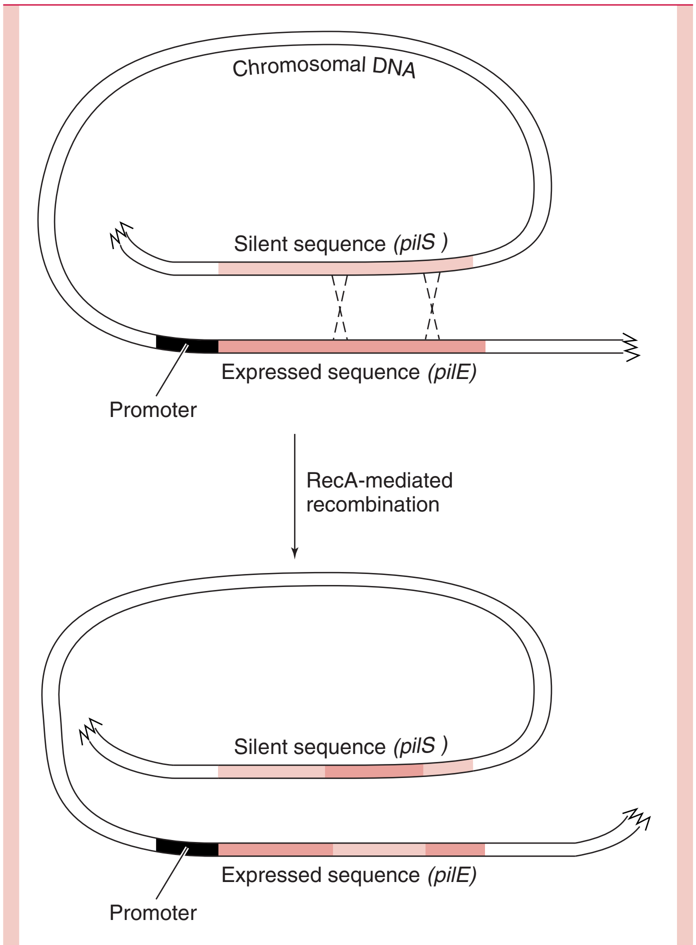


FIGURE 4–10
Schematic diagram illustrating phase variation of surface antigens in *Neisseria gonorrhoeae* by the cassette mode of gene regulation involving recombination at an expression site.

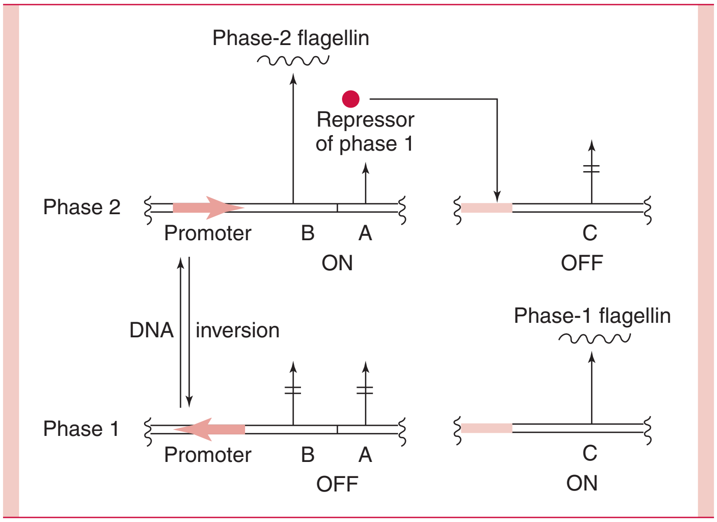


FIGURE 4–11
Schematic diagram illustrating alternate expression of flagellins in *Salmonella* by a genetic switch composed of an invertible element. (*Adapted from Macnab RM. Flagella and motility. In: Neidhardt FC, Curtiss R III, Ingraham JL, et al,* Escherichia coli and Salmonella: Cellular and Molecular Biology, Washington DC: ASM Press; 1966. pp 123–145.)

lacks its own promoter, depends on a promoter within the invertible element. In one orientation, this promoter can initiate transcription of the B gene; in the other orientation transcription, if it starts, proceeds in the opposite direction, and the B gene is silent, allowing the C gene to work. In this manner, excision of the invertible element and its reinsertion at the same site but in the opposite orientation lead to a shift from one flagellar form to the other (ie, to antigenic phase variation). The invertible element encodes its own site-specific **recombinase enzyme** that catalyzes the inversion in response to currently unknown signals. A similar situation exists in *E. coli,* where a 314-bp invertible segment containing a promoter controls transcription of the adjacent, promoter-less *fimA* gene. This gene encodes the structural protein for type 1 (common) pili, which function as an adhesin in mediating the binding of *E. coli* to eukaryotic cells, thereby aiding in the early stages of tissue colonization by these bacteria.

It is believed that antigenic variation mediated by these site-specific transpositional rearrangements provides a selective advantage to the bacteria in allowing invading populations to include individuals that can escape the developing immune response of the host and thus continue the infectious process. Similar strategies are used by some eukaryotic parasites of humans, notably the trypanosomes (see Chapter 54).

## MORE ABOUT BACTERIAL PLASMIDS

One of the unanticipated features of microbial genetics has been the revelation that many virulence factors and much clinically significant resistance to antibiotics are the result of the activities not of bacterial chromosomal genes but of the accessory genomes present in plasmids. In a certain sense, the health professional treating infectious disease is frequently coping with autonomous self-replicating DNA molecules. Many of the properties of plasmids have already been touched on, but the information is now consolidated and considered in more detail.

### General Properties and Varieties of Plasmids

We have already encountered plasmids in our consideration of conjugation. To recap, plasmids are ubiquitous extrachromosomal elements composed of double-stranded DNA that typically is circular (Fig 4–12). (Linear plasmids occur in medically relevant strains of *Borrelia*.) A single organism can harbor several distinct plasmids. Like the chromosome, they have the property of governing their own replication by means of special sequences and regulatory proteins, including a genetic region called *ori* (origin of replication) at which specific proteins initiate replication. Any DNA molecule that is self-reproducing, including all plasmids as well as the bacterial chromosome, is said to be a **replicon.**

Plasmids are replicons found in most bacterial species in nature

Plasmids vary greatly in size, in the mode of control of their replication, and in the number and kinds of genes they carry. Naturally occurring plasmids range from less than

Plasmids very greatly in size and control of replication

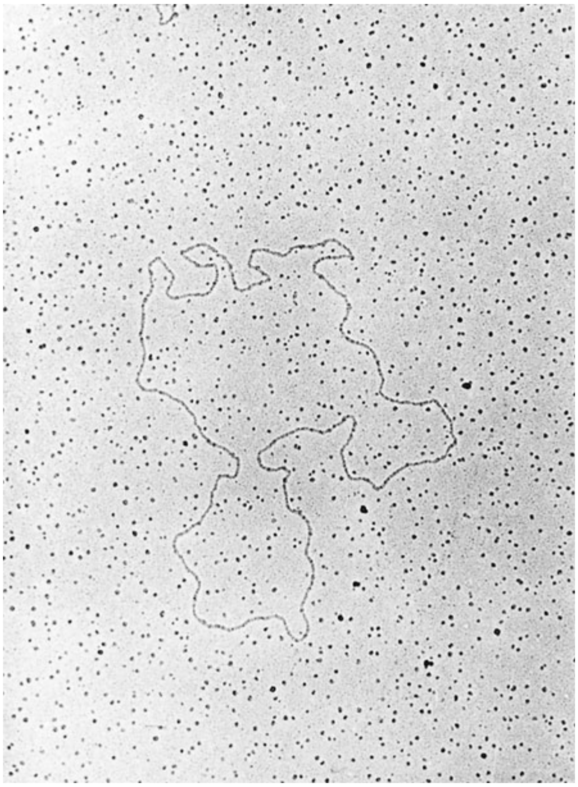

FIGURE 4–12
Electron micrograph of an R plasmid from *Escherichia coli*. The plasmid is 64 megadaltons and contains about 40 kilobase pairs. (*Courtesy of Dr. Jorge H. Crosa.*)

5 million to more than 100 million daltons, but even the largest are only a few percent of the size of the bacterial chromosome. The number of molecules of a given plasmid that is present in a cell, called the **copy number,** varies greatly among different plasmids, from only a few molecules per cell to dozens of molecules per cell. In general, small plasmids tend to be represented by more copies per cell.

Small plasmids are often present in multiple copies per cell

Conjugal transfer is an important property of those plasmids that possess the complex of *tra* genes, but even nonconjugative plasmids can transfer to some extent to other cells as a result of mobilization by conjugative plasmids. Some plasmids, again including the F factor, can replicate either autonomously or as a segment of DNA integrated into the chromosome. These are sometimes termed **episomes.** Certain prophages can exist as plasmids, but most plasmids are not viruses, because at no point of their life cycle do they exist as a free viral particle (**virion**). Most plasmids show little or no DNA homology with the chromosome and can, in this sense, be regarded as foreign to the cell.

Conjugative plasmids can facilitate transfer of nonconjugatives

Some plasmids, called episomes, can integrate and replicate with the chromosome

Most plasmids are nonhomologous with the host cell chromosome

Plasmids usually include a number of genes in addition to those required for their replication and transfer to other cells. The variety of cellular properties associated with plasmids is very great and includes fertility (the capacity for gene transfer by conjugation), production of toxins, production of pili and other adhesins, resistance to antimicrobics and other toxic chemicals, production of bacteriocins (toxic proteins that kill some other bacteria), production of siderophores for scavenging $Fe^{3+}$, and production of certain catabolic enzymes important in biodegradation of organic residues.

Bacterial adaptation to environment depends heavily on properties encoded by plasmids

Many plasmid genes promote survival and colonization and hence pathogenesis

On the other hand, plasmids can add a small metabolic burden to the cell, and in many cases, a slightly reduced growth rate results. Thus, under conditions of laboratory cultivation where the properties coded by the plasmid are not required, there is a tendency for **curing** of a strain to occur, because the progeny cells that have not acquired a plasmid (or have lost it) have a selective advantage during prolonged growth and subculture. Conversely, where the property conferred by the plasmid is advantageous (eg, in the presence of the antimicrobic to which the plasmid determines resistance), selective pressure favors the plasmid-carrying strain.

In absence of selection pressure for their properties, plasmids may be lost due to spontaneous curing

Although plasmids are central to infectious disease and have been studied for decades, their origin remains uncertain. They could possibly be descendants of bacterial viruses that evolved a sophisticated means of self-transfer by conjugation and then lost their unneeded protein capsid. Alternatively, they may have evolved as separated parts of a bacterial chromosome that could provide both the means for genetic exchange and a way to amplify certain genes of special value in a particular environment (eg, coding for an adhesin) or to dispense with them where they are superfluous.

Plasmids could have any of several theoretically possible origins

A great many bacterial plasmids are known. Some show similarity with each other in nucleotide sequence; thus, plasmids can be classified by their degree of apparent relatedness. Unrelated plasmids can coexist within a single cell, but closely related plasmids become segregated during cell division and eventually all but one are eliminated. For this reason, a group of closely related plasmids that exclude each other are referred to as an **incompatibility group.**

Cells may harbor more than one plasmid type provided they are unrelated to each other

## R Plasmids

Plasmids that include genes conferring resistance to antimicrobics are of great significance to medicine. They are termed **R plasmids** or **R factors** (**resistance factors**). The genes responsible for resistance usually code for enzymes that inactivate antimicrobics or reduce the cell's permeability to them. In contrast, resistance conferred by chromosomal mutation usually involves modification of the target of the antimicrobics (eg, RNA polymerase or the ribosome).

Plasmids confer resistance by inactivating antimicrobics or reducing their entry

R plasmids occupy center stage in approaches to chemotherapy because of the constellation of properties they possess. Those of Gram-negative bacteria can be transmitted across species boundaries and, at lower frequency, even between genera. Many encode resistance to several antimicrobics and can thus spread multiple resistance through a diverse microbial population under selective pressure of only one of those agents to which they confer resistance. Nonpathogenic bacteria can serve as a natural reservoir of resistance determinants on plasmids that are available for spread to pathogens.

R plasmids can encode and transfer multiresistance

R plasmids evolve rapidly and can easily acquire additional resistance-determining genes from fusion with other plasmids or acquisition of transposons. Many have the capability of amplifying the number of copies of their resistance genes either by gene duplications within each plasmid or by increasing the number of plasmids (copy number) per cell. By these means resistance can be achieved to very high concentrations of the antimicrobic. One process of gene amplification is based on the ability of some conjugative plasmids to dissociate their components into two plasmids, one (called the **resistance transfer factor**) containing genes for replication and for transfer and another (called the **resistance** or **r determinant**) containing genes for replication and for resistance. Subsequent relaxed replication of the r determinant expands the cell's capacity to produce the resistance-conferring enzyme (Fig 4–13).

Resistance genes can be acquired by plasmids from transposons or through plasmid fusion

Resistance genes can be amplified by increasing copy number

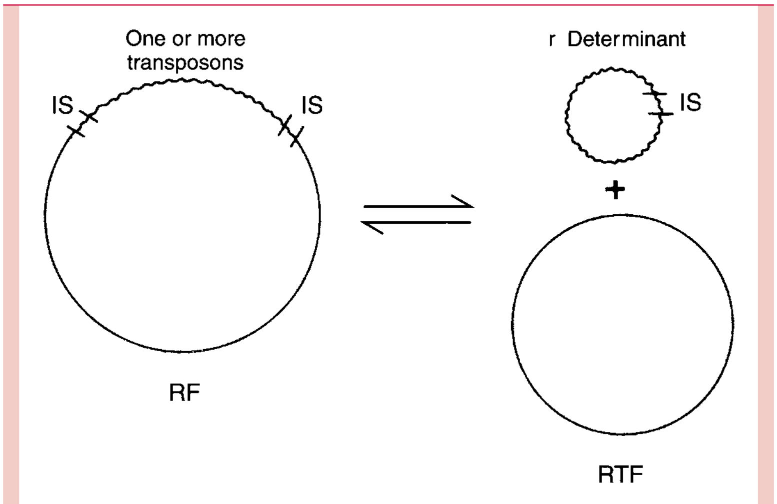


FIGURE 4–13
Structure and dissociation of an R-factor (RF) plasmid. The RF plasmid is shown with its two components: the r determinant, which contains one or more genes for antibiotic resistance (frequently present as transposons), and the resistance transfer factor (RTF), which contains the genes necessary for replication of the plasmid and its transfer to other cells. IS, insertion resistance.

Resistance spread is facilitated by transposition of plasmid genes for resistance

Widespread use of antimicrobics selects formation and spread of R plasmids

Over the past three decades, many of the molecular feats of R plasmids have been explained on the basis of known genetic and evolutionary mechanisms. The discovery of transposable elements (insertion sequences and transposons) and their properties provides an explanation for many of these phenomena. Most plasmids, and all R factors, contain many IS elements and transposons. In fact, virtually all the resistance determinant genes on plasmids are present as transposons. As a result, these genes can be amplified by tandem duplications on the plasmid and can hop to other plasmids (or to the bacterial chromosome) in the same cell. Combined with the natural properties of many plasmids to transfer themselves by conjugation (even between dissimilar bacterial species), the rapid evolutionary development of multiple drug resistance plasmids and their spread through populations of pathogenic bacteria during the past three decades can be seen as a predictable result of natural selection resulting from the widespread and intensive use of antimicrobics in human and veterinary medicine (see Chapter 14).

Resistance genes preexisted antimicrobic use in medicine

The properties of transposons can explain the present-day ubiquity and mobility of resistance genes but not their origin. Two facts help point to at least a direction in which to search for an answer. First, R plasmids carrying the genes encoding antimicrobic-inactivating enzymes have been found in bacterial cultures preserved by lyophilization (freeze-drying) since before the era of antimicrobic therapy; an accelerated evolutionary development need not be invoked. Second, the enzymes themselves are remarkably similar to those found in certain bacteria (*Streptomyces* spp) that produce many clinically useful antimicrobics. Perhaps a long time ago there was a cross-genus transfer of genetic information (by transformation?) that became stabilized on plasmids under the selection pressure of an antimicrobic released into the environment under natural conditions.

## Detection of Plasmids

Plasmid involvement is implicated by rapid transfer of multiresistance

A number of physical, morphologic, and functional tests can be used to reveal the presence of plasmids in a bacterial population. The rapid transfer of characteristics, such as resistance to antimicrobics, from strain to strain or, alternatively, the rapid loss of such traits is a hallmark of plasmid-encoded characteristics. When several genetically distinct characteristics are transferred simultaneously in the laboratory into cells known not to have possessed them previously, the evidence is very strong that a plasmid is responsible. Plasmids, including nonconjugative plasmids and those coding for no presently known trait, can be

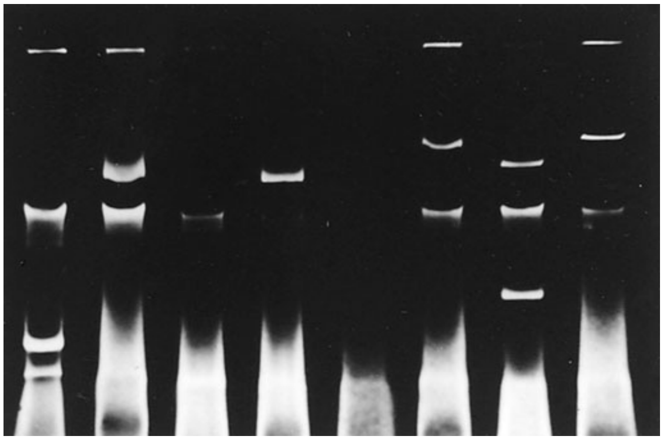

FIGURE 4–14
Agarose gel electrophoresis of various strains of staphylococci isolated from patients in a large metropolitan hospital. Each vertical lane displays the DNA of a separate isolate. The sharp bands visible in the upper half of most lanes are plasmids. The broad smear of DNA in the lower half is chromosomal DNA. The results illustrate the prevalence of multiple plasmids in freshly isolated bacterial strains. Most isolates contain more than one plasmid. (*Courtesy of Dr. D. R. Schaberg, University of Michigan*.)

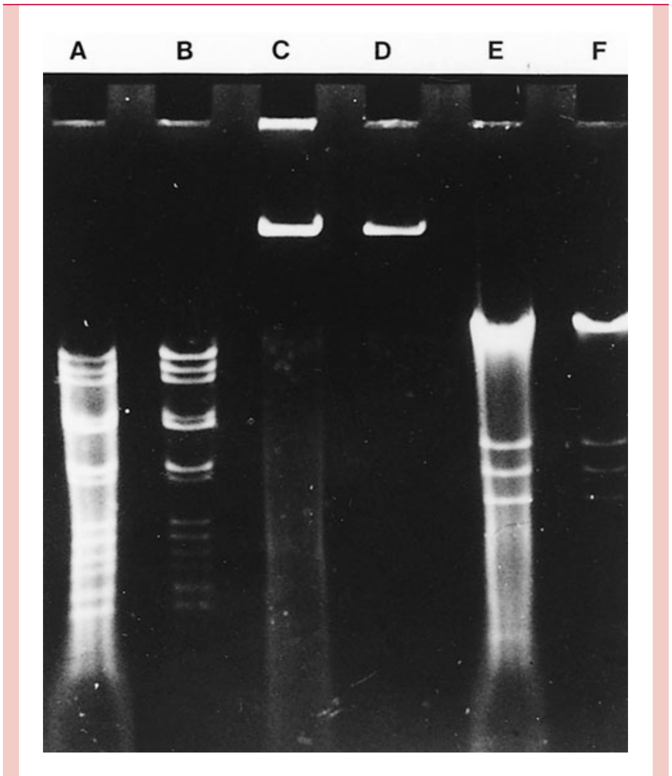


FIGURE 4–15
Use of agarose gel electrophoresis in molecular epidemiology. During an outbreak of bacteremia in infants in a neonatal intensive care unit, strains of *Klebsiella aerogenes* and *Enterobacter cloacae* were isolated that harbored R-factor plasmids of similar electrophoretic mobility and conferred resistance to some aminoglycosides, ampicillin, and chloramphenicol. To learn if an identical plasmid had established itself in both bacterial species, a restriction digest analysis was performed and the products were separated by electrophoresis. Lanes C and D display the intact plasmid DNA isolated from *K. aerogenes* and *E. cloacae,* respectively. Lanes A and B display the fragments produced by the action of the restriction enzyme *Bam*HI on the plasmids, and lanes E and F display the fragments produced by the restriction enzyme *Eco*RI. For each pair of treated samples, the plasmid DNA from *K. aerogenes* is on the left (ie, lanes A and E). The identical restriction patterns make it almost certain that the plasmids from the two bacterial species are identical, and raise the possibility that the epidemic itself was caused by the chance introduction and spread of this R plasmid. (*Kindly provided by Dr. D. R. Schaberg, University of Michigan.*)

demonstrated directly by agarose gel electrophoresis. These methods and their diagnostic application are discussed in Chapter 15. Electron microscopy can also be used to visualize plasmids, to measure the length of their DNA, and to see the forms they take on hybridization to other nucleic acid molecules (see Fig 4–12).

Plasmid DNAs are separable electrophoretically

Bacterial plasmids, including R factors, have become valuable markers for comparing closely related strains of bacteria in epidemiologic studies. In outbreaks, spread of an epidemic strain can sometimes be followed more easily and more accurately by monitoring the profile of plasmids carried in strains isolated from different patients than by using traditional typing methods (Fig 4–14). This approach is particularly useful in studying outbreaks of nosocomial (hospital-acquired) infections. Likewise, the spread of an R plasmid between different species can be followed by showing that they carry an identical plasmid conferring the same pattern of antimicrobic resistance. The plasmid comparison can be carried one step further in specificity by cutting the plasmid DNA with specific restriction endonucleases (see next section) and examining the resulting fragments by agarose gel electrophoresis (Fig 4–15). Variations of this procedure enable even the spread of specific genes among a variety of plasmids to be detected.

Tracing plasmids is valuable in the epidemiology of disease outbreaks

Endonuclease digestion is useful in comparing plasmids

## BACTERIAL CLASSIFICATION

Bacteria are classified into genera and species according to a binomial Linnean scheme similar to that used for higher organisms. For example, in the case of *Staphylococcus aureus, Staphylococcus* is the name of the **genus** and *aureus* is the **species** designation. Some genera with common characteristics are further grouped into **families.** However, bacterial classification has posed many problems. Morphologic descriptors are not as abundant as in higher plants and animals, there is little readily interpreted fossil record to help establish phylogeny, and there is no elaborate developmental process (ontogeny) to recapitulate the evolutionary path from ancestral forms (phylogeny). These problems are minor compared with others: bacteria mutate and evolve rapidly, they reproduce asexually, and they exchange genetic material over wide boundaries. The single most important test of species, the ability of individuals within a species to reproduce sexually by mating and exchanging genetic material, cannot be applied to bacteria. As a result, bacterial taxonomy developed pragmatically by determining multiple characteristics and weighting them according to which seemed most fundamental; for example, shape, spore formation, Gram reaction, aerobic or anaerobic growth, and temperature for growth were given special weighting in defining genera. Such properties as ability to ferment particular carbohydrates, production of specific enzymes and toxins, and antigenic composition of cell surface components were often used in defining species. As presented in Chapter 15, such properties and their weighting continue to be of central importance in identification of unknown isolates in the clinical laboratory, and the use of determinative keys is based on the concept of such weighted characteristics. These approaches are much less sound in establishing taxonomic relationships based on phylogenetic principles.

Weighted classification schemes are more valuable for identification than for taxonomy

### New Taxonomic Methods

The recognition that sound taxonomy ought to be based on the genetic similarity of organisms and to reflect their phylogenetic **relatedness** has led in recent years to the use of new methods and new principles in taxonomy. The first approach was to apply **Adansonian** or **numeric taxonomy,** which gives equal weighting to a large number of independent characteristics and allocates bacteria to groups according to the proportion of shared characteristics as determined statistically. Theoretically, a significant correspondence of a large number of phenotypic characteristics could be considered to reflect genetic relatedness.

Degrees of genetic similarity are important for sound taxonomy

A more direct approach available in recent years involves analysis of chromosomal DNA. Analysis can be somewhat crude, such as the overall ratio of A–T to G–C base pairs; differences of greater than 10% in G–C content are taken to indicate unrelatedness, but closely similar content does not imply relatedness. Closer relationships can be assessed by determining base sequence similarity, as by DNA–DNA hybridization, in which single strands of DNA from one organism are allowed to anneal with single strands of another. Some clinical laboratory tests have been devised based on the ability of DNA from a reference strain to undergo homologous recombination with DNA from an unknown isolate (see Chapter 15). However, overwhelmingly the molecular genetic technique that is introducing the greatest change in infectious disease diagnosis is the comparison of nucleotide sequences of genes highly conserved in evolution, such as 16 S ribosomal RNA genes. So successful have been the deductions of phylogenetic relatedness based on these sequences that the absence of a fossil record is now regarded as insignificant. Part of the excitement in this field is that the use of polymerase chain reaction to amplify the DNA of cells has made it possible to identify even infectious organisms that cannot be cultivated in the laboratory.

Phylogentic relationships are assuming greater significance as the result of DNA sequence analysis

### Genomic Approaches to Virulence

The most startling recent advance in medical microbiology is indicated by the fact that in the few years since the printing of the previous edition of this book, the complete nucleotide sequences of the genomes of several dozen medically significant bacteria have been determined. Furthermore, advances in the technology of DNA sequencing promise the rapid determination of many more genomes in the next few years. It is difficult to overstate the significance of the present situation. First, comparison of virulent with nonvirulent species of closely related bacteria is providing means to identify virulence genes, that is, genes responsible for the disease-producing capability of these bacteria. Second, thanks to the sequence information, the products of these genes can readily be produced and studied, and mutants can be prepared for genetic and functional analysis. Among the genes being discovered in this way are many organisms of hitherto unknown virulence, providing new information about the many molecular processes involved in pathogenesis. Third, new information on virulence factors and how they work is suggesting new, rational design of therapeutic and prophylactic agents to replace our current overreliance on natural antimicrobics and their chemical derivatives.

Finally, detailed genomic analysis of pathogens involves suggesting pathways of the evolution of important human and animal pathogens. Already, molecular genetic studies have uncovered the existence of pathogenicity islands (PAIs) within genomes—that is, groups of adjacent genes that encode functions important for colonization, invasion, avoidance of host defenses, and production of tissue damage. These PAIs exist not only within the chromosome of pathogens but also within the plasmids that assist in conferring virulence properties on the bacteria. As described in Chapter 10, analysis of PAIs provides important clues to the origin of these gene clusters and to their transmission between species. This should be a fertile area for understanding the evolution of pathogens.

Genome sequences will greatly accelerate studies on infectious disease processes, their evolution, and their successful management

## POPULATION GENETICS OF PATHOGENS

One of the discoveries to come from the application of molecular diagnostic tools to infectious diseases is the clonal nature of many infectious diseases. That is, over long periods and large geographic distances, the organisms of a given species isolated from clinical samples tend to be so similar in chromosomal genetic makeup (and in their plasmid profiles) that one is forced to envision that a clone of bacteria descended from a relatively recent common ancestor is responsible for all or most of the disease incidence. This evidence comes partly from studies of **plasmid profiles,** but mostly it is a conclusion drawn by examining the specific alleles of various genes present in a population of cells using the technique of **multilocus enzyme electrophoresis.** Differences in electrophoretic migration are used to detect subtle differences in amino acid sequence in a battery of two to three dozen different enzymes. The results have been striking. For example, isolates of *Bordetella pertussis* from the United States represent a single clone, whereas in Japan there is a slightly different clone. Another study has determined that only 11 multilocus genotypes (clones) of *Neisseria meningitidis* have been responsible for the major epidemics of serogroup A organisms worldwide over the past 60 years. These discoveries provide an entirely new method for study of the epidemiology of infectious disease.

Natural populations of many pathogens are proving to have a clonal structure

In some cases single clones are responsible for geographically widespread disease

## ADDITIONAL READING

Finlay BB, Falkow S. Common themes in microbial pathogenicity revisited. *Microbiol Mol Biol Rev* 1997;61:136–169. An interesting and highly readable account of the major contemporary themes in microbial pathogenicity.

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PART II

# Biology of Viruses

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CHAPTER 5

# Viral Structure

JAMES J. CHAMPOUX

A virus is a set of genes, composed of either DNA or RNA, packaged in a protein-containing coat. The resulting particle is called a **virion.** Viruses that infect humans are considered along with the general class of animal viruses; viruses that infect bacteria are referred to as bacteriophages, or phages for short. Virus reproduction requires that a virus particle infect a cell and program the cellular machinery to synthesize the constituents required for the assembly of new virions. Thus, a virus is considered an intracellular parasite. The infected host cell may produce hundreds to hundreds of thousands of new virions and usually dies. Tissue damage as a result of cell death accounts for the pathology of many viral diseases in humans. In some cases, the infected cells survive, resulting in persistent virus production and a chronic infection that can remain asymptomatic, produce a chronic disease state, or lead to relapse of an infection.

A virus is an intracellular parasite composed of DNA or RNA and a protein coat

In some circumstances, a virus fails to reproduce itself and instead enters a latent state (called **lysogeny** in the case of bacteriophages), from which there is the potential for reactivation at a later time. A possible consequence of the presence of viral genes in a latent state is a new genotype for the cell. Some determinants of bacterial virulence and some malignancies of animal cells are examples of the genetic effects of latent viruses. Apparently vertebrates have had to coexist with viruses for a long time because they have evolved the special nonspecific interferon system, which operates in conjunction with the highly specific immune system to combat virus infections.

Instead of reproducing, the virus may enter a latent state from which it can later be activated

In the discussion to follow, the biological and genetic bases for these phenomena are presented; three themes are emphasized.

1. Different viruses can have very different genetic structures, and this diversity is reflected in their replicative strategies.
2. Because of their small size, viruses have achieved a very high degree of genetic economy.
3. Viruses depend to a great extent on host cell functions and, therefore, are difficult to combat medically. They do exhibit unique steps in their replicative cycles that are potential targets for antiviral therapy.

## VIRION SIZE AND DESIGN

Viruses are approximately 100- to 1000-fold smaller than the cells they infect. The smallest viruses (parvoviruses) are approximately 20 nm in diameter (1 nm = $10^{-9}$ m), whereas the largest animal viruses (poxviruses) have a diameter of approximately 300 nm

Viral size ranges from 20 to 300 nm

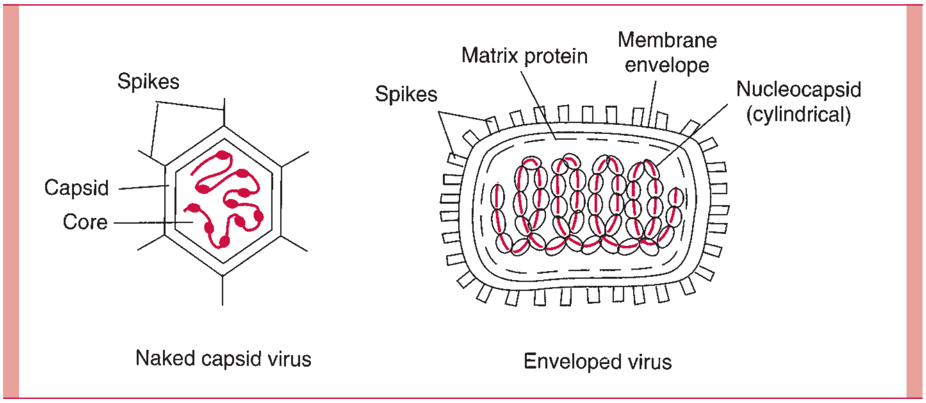


FIGURE 5–1
Schematic drawing of two basic types of virions.

and overlap the size of the smallest bacterial cells (*Chlamydia* and *Mycoplasma*). Therefore, viruses generally pass through filters designed to trap bacteria, and this property can, in principle, be used as evidence of a viral etiology.

Naked capsid viruses have a nucleic acid genome within a protein shell

Enveloped viruses have a nucleocapsid of nucleic acid complexed to protein

Viruses often have surface protrusions

The basic design of all viruses places the nucleic acid genome on the inside of a protein shell called a **capsid.** Some animal viruses are further packaged into a lipid membrane, or **envelope,** which is usually acquired from the cytoplasmic membrane of the infected cell during egress from the cell. Viruses that are not enveloped have a defined external capsid and are referred to as **naked capsid viruses.** The genomes of enveloped viruses form a protein complex and a structure called a **nucleocapsid,** which is often surrounded by a matrix protein that serves as a bridge between the nucleocapsid and the inside of the viral membrane. Protein or glycoprotein structures called **spikes,** which often protrude from the surface of virus particles, are involved in the initial contact with cells. These basic design features are illustrated schematically in Figure 5–1 as well as in the electron micrographs in Figures 5–2 and 5–3.

Two basic shapes: cylindrical and spherical

The protein shell forming the capsid or the nucleocapsid assumes one of two basic shapes: cylindrical or spherical. Some of the more complex bacteriophages combine these two basic shapes. Examples of these three structural categories can be seen in the electron micrographs in Figure 5–2.

Outer shell is protective and aids in entry and packaging

The capsid or envelope of viruses functions (1) to protect the nucleic acid genome from damage during the extracellular passage of the virus from one cell to another, (2) to aid in the process of entry into the cell, and (3) in some cases to package enzymes essential for the early steps of the infection process.

Nucleic acid must be condensed during virion assembly

In general, the nucleic acid genome of a virus is hundreds of times longer than the longest dimension of the complete virion. It follows that the viral genome must be extensively condensed during the process of virion assembly. For naked capsid viruses, this condensation is achieved by the association of the nucleic acid with basic proteins to form what is called the **core** of the virus (see Fig 5–1). The core proteins are usually encoded by the virus, but in the case of some DNA-containing animal viruses, the basic proteins are histones scavenged from the host cell. For enveloped viruses, the formation of the nucleocapsid serves to condense the nucleic acid genome.

Plant viroids are infectious RNA molecules

Prions may cause spongiform encephalopathies

Two classes of infectious agents exist that are structurally simpler than viruses. **Viroids** are infectious circular RNA molecules that lack protein shells; they are responsible for a variety of plant diseases. Hepatitis delta, an infectious agent sometimes found in association with hepatitis B virus, appears to share many properties with the viroids. **Prions,** which apparently lack any genes and are composed only of protein, are agents that appear to be responsible for some transmissible and inherited spongiform encephalopathies such as scrapie in sheep; bovine spongiform encephalopathy in cattle; and kuru, Creutzfeldt-Jakob disease, and Gerstmann-Sträussler-Scheinker syndrome in humans.

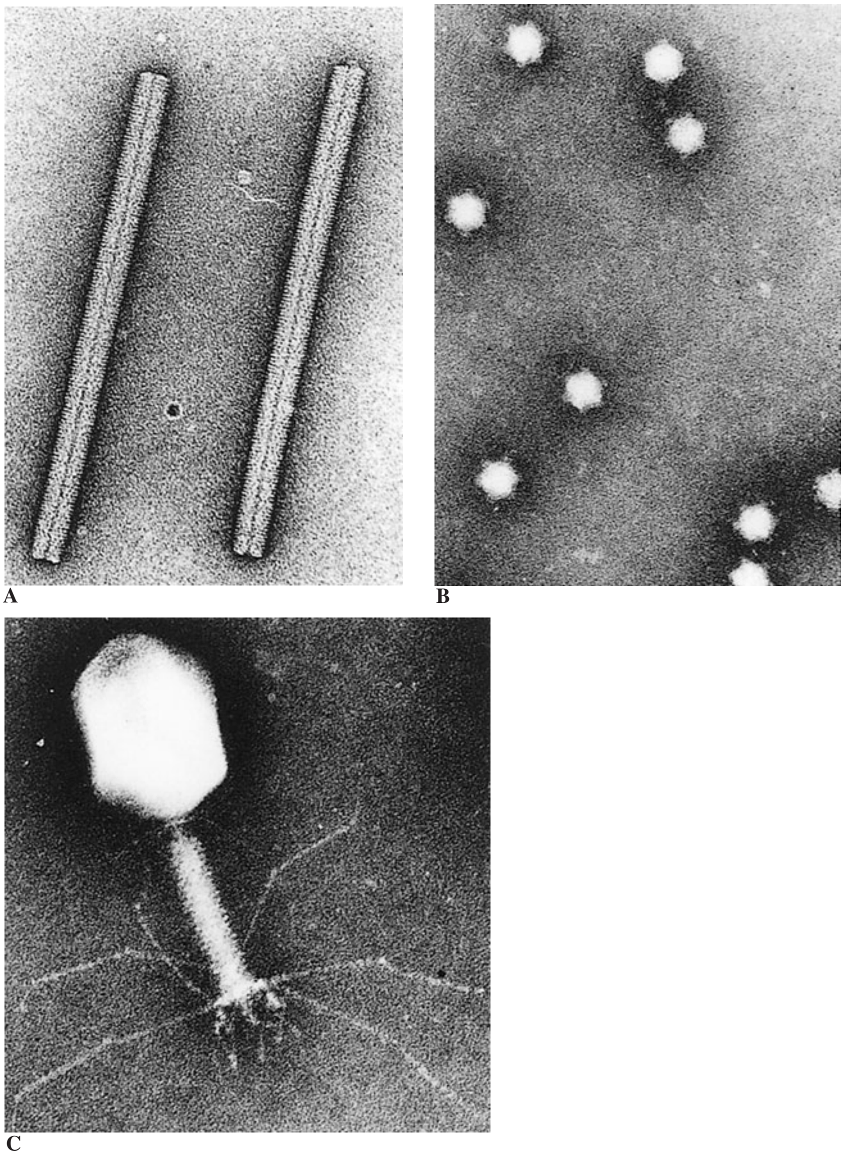


FIGURE 5–2
Three basic virus designs:
**A.** Tobacco mosaic virus.
**B.** Bacteriophage $\phi$X174.
**C.** Bacteriophage T4. (*Kindly provided by Dr. Robley C. Williams.*)

## GENOME STRUCTURE

Structural diversity among the viruses is most obvious when the makeup of viral genomes is considered. Genomes can be made of RNA or DNA and be either double stranded or single stranded. For viruses with single-stranded genomes, the nucleic acid can be either of the same polarity (indicated by a +) or of a different polarity (−) from that of the viral mRNA produced during infection. In the case of adeno-associated viruses, the particles are a mixture: about half contain (+)DNA; the other half contain (−)DNA. The arenaviruses and bunyaviruses are unusual in having an RNA genome, part of which has the same polarity as the mRNA and part of which is complementary to the corresponding mRNA.

DNA or RNA genomes may be single or double stranded

Both linear and circular genomes are known. Whereas the genomes of most viruses are composed of a single nucleic acid molecule, in some cases several pieces of nucleic acid constitute the complete genome. Such viruses are said to have **segmented** genomes. One virus class (retroviruses) carries two identical copies of its genome and is therefore diploid. A few viral genomes (picornaviruses, hepatitis B virus, and adenoviruses) contain covalently attached protein on the ends of the DNA or RNA chains that are remnants of the replication process.

Genomes may be linear or circular

Some genomes are segmented

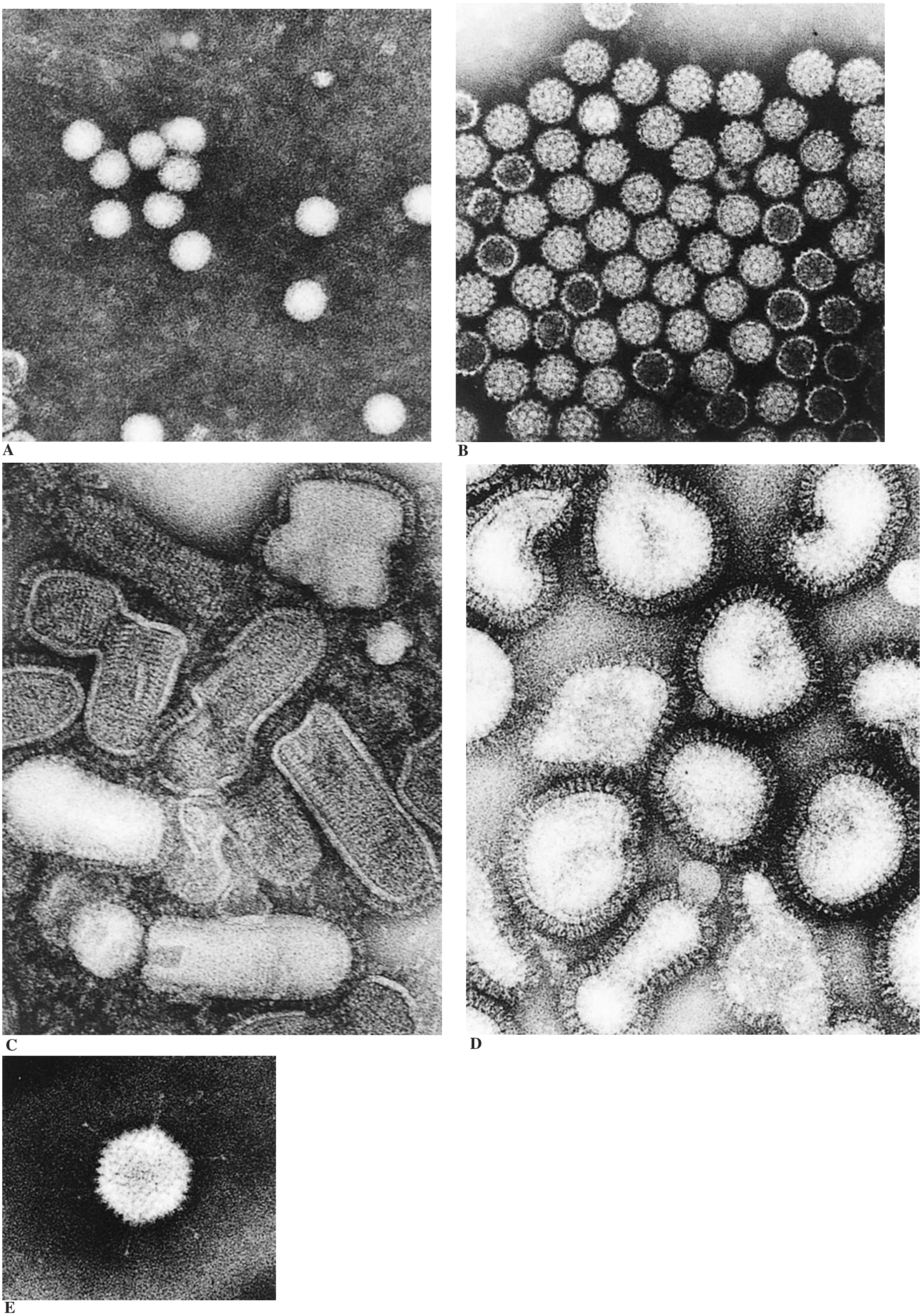


FIGURE 5–3

Representative animal viruses: **A.** Poliovirus. **B.** Simian virus 40. **C.** Vesicular stomatitis virus. **D.** Influenza virus. **E.** Adenovirus. (*Kindly provided by Dr. Robley C. Williams.*)

## CAPSID STRUCTURE

### Subunit Structure of Capsids

The capsids or nucleocapsids of all viruses are composed of many copies of one or at most several different kinds of protein subunits. This fact follows from two fundamental considerations. First, all viruses code for their own capsid proteins, and even if the entire coding capacity of the genome were to be used to specify a single giant capsid protein, the protein would not be large enough to enclose the nucleic acid genome. Thus, multiple protein copies are needed, and, in fact, the simplest spherical virus contains 60 identical protein subunits. Second, viruses are such highly symmetric structures that it is not uncommon to visualize naked capsid viruses in the electron microscope as a crystalline array (eg, simian virus 40 in Fig 5–3B). The simplest way to construct a regular symmetrical structure out of irregular protein subunits is to follow the rules of crystallography and form an aggregate involving many identical copies of the subunits, where each subunit bears the same relationship to its neighbors as every other subunit.

Capsids and nucleocapsids are composed of multiple copies of protein molecule(s) in crystalline array

The presence of many identical protein subunits in viral capsids or the existence of many identical spikes in the membrane of enveloped viruses has important implications for adsorption, hemagglutination, and recognition of viruses by neutralizing antibodies (see Chapter 6).

### Cylindrical Architecture

A cylindrical shape is the simplest structure for a capsid or a nucleocapsid. The first virus to be crystallized and studied in structural detail was a plant pathogen, tobacco mosaic virus (TMV) (see Fig 5–2A). The capsid of TMV is shaped like a rod or a cylinder, with the RNA genome wound in a helix inside it. The capsid is composed of multiple copies of a single kind of protein subunit arranged in a close-packed helix, which places every subunit in the same microenvironment. Because of the helical arrangement of the subunits, viruses that have this type of design are often said to have helical symmetry. Although less is known about the architecture of animal viruses with helical symmetry, it is likely their structures follow the same general pattern as TMV. Thus, the nucleocapsids of influenza, measles, mumps, rabies, and poxviruses (Table 5–1) are probably constructed with a helical arrangement of protein subunits in close association with the nucleic acid genome.

Cylindrical viruses have capsid protein molecules arranged in a helix

### Spherical Architecture

The construction of a spherically shaped virus similarly involves the packing together of many identical subunits, but in this case the subunits are placed on the surface of a geometric solid called an **icosahedron.** An icosahedron has 12 vertices, 30 sides, and 20 triangular faces (Fig 5–4). Because the icosahedron belongs to the symmetry group that crystallographers refer to as cubic, spherically shaped viruses are said to have cubic symmetry. (Note that the term **cubic,** as used in this context, has nothing to do with the more familiar shape called the cube.)

Spherical viruses exhibit icosahedral symmetry

When viewed in the electron microscope, many naked capsid viruses and some nucleocapsids appear as spherical particles with a surface topology that makes it appear that they are constructed of identical ball-shaped subunits (see Fig 5–3B and E). These visible structures are referred to as **morphological subunits,** or **capsomeres.** A capsomere is generally composed of either five or six individual protein molecules, each one referred to as a **structural subunit,** or **protomer.** In the simplest virus with cubic symmetry, five protomers are placed at each one of the 12 vertices of the icosahedron as shown in Figure 5–4 to form a capsomere called a **pentamer.** In this case, the capsid is composed of 12 pentamers, or a total of 60 protomers. It should be noted that as in the case of helical symmetry, this arrangement places every protomer in the same microenvironment as every other protomer.

Capsomeres are surface structures composed of five or six protein molecules

To accommodate the larger cavity required by viruses with large genomes, the capsids contain many more protomers. These viruses are based on a variation of the basic icosahedron in which the construction involves a mixture of pentamers and hexamers instead of only pentamers. A detailed description of this higher level of virus structure is beyond the scope of this text.

TABLE 5–1

**Classification of RNA Animal Viruses**

| Family | Virion Structure | Genome Structure and Molecular Weight | Representative Members |
|---|---|---|---|
| Hepatitis δ | Cubic, enveloped | ss circular (−) ($6 \times 10^5$) | Human hepatitis δ virus |
| Picornaviruses | Cubic, naked | ss linear (+) ($2–3 \times 10^6$); protein attached | Human enteroviruses: poliovirus, coxsackievirus, echovirus; rhinoviruses; bovine foot-and-mouth disease virus; hepatitis A |
| Arenaviruses | Helical, enveloped | 2 ss linear segments (+/−) ($3 \times 10^6$) | Lassa virus; lymphocytic choriomeningitis virus of mice |
| Caliciviruses | Cubic, naked | ss linear (+) ($2.6 \times 10^6$) | Vesicular exanthema virus, Norwalk-like viruses of humans |
| Rhabdoviruses | Helical, enveloped | ss linear (–) ($3–4 \times 10^6$) | Rabies virus; bovine vesicular stomatitis virus |
| Retroviruses | Cubic, enveloped | ss linear (+), diploid ($3–4 \times 10^6$) | RNA tumor viruses of mice, birds, and cats; visna virus of sheep; human immunodeficiency viruses (human T-cell leukemia and acquired immunodeficiency syndrome) |
| Togaviruses | Cubic, enveloped | ss linear (+) ($4 \times 10^6$) | Alphaviruses: Sindbis virus and Semliki Forest virus; flaviviruses: dengue virus and yellow fever virus; rubella virus; mucosal disease virus |
| Orthomyxoviruses | Helical, enveloped | 8 ss linear segments (−) ($5 \times 10^6$) | Type A, B, and C influenza viruses of humans, swine, and horses |
| Coronaviruses | Helical, enveloped | ss linear (+) ($5–6 \times 10^6$) | Respiratory viruses of humans; calf diarrhea virus; swine enteric virus; mouse hepatitis virus |
| Filoviruses | Helical, enveloped | ss linear (−) ($5 \times 10^6$) | Marburg and Ebola viruses |
| Bunyaviruses | Helical, enveloped | 3 ss linear segments (+/−) ($6 \times 10^6$) | Rift Valley fever virus; bunyamwera virus; hantavirus |
| Paramyxoviruses | Helical, enveloped | ss linear (−) ($6–8 \times 10^6$) | Mumps; measles; Newcastle disease virus; canine distemper virus |
| Reoviruses | Cubic, naked | 10 ds linear segments ($15 \times 10^6$) | Human reoviruses; orbiviruses; Colorado tick fever virus; African horse sickness virus; human rotaviruses |

*Abbreviations:* ss, single stranded; ds, double stranded.

## Special Surface Structures

Surface structures are important in adsorption and penetration

Many viruses have structures that protrude from the surface of the virion. In virtually every case these structures are important for the two earliest steps of infection, adsorption and penetration. The most dramatic example of such a structure is the tail of some bacteriophages (see Fig 5–2C), which, as described in Chapter 6, acts as a channel for the transfer of the genome into the cell. Other examples of surface structures include the spikes of adenovirus (see Fig 5–3E) and the glycoprotein spikes found in the membrane of enveloped viruses (see influenza virus in Fig 5–3D). Even viruses without obvious

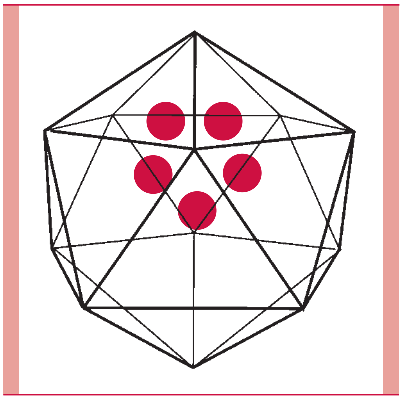

FIGURE 5–4
Diagram of an icosahedron showing 12 vertices, 20 faces, and 30 sides. The colored balls indicate the position of protomers forming a pentamer on the icosahedron.

surface extensions probably contain short projections, which, like the more obvious spikes, are involved in the specific binding of the virus to the cell surface (see Chapter 6).

## Classification of Viruses

Tables 5–1 and 5–2 present a classification scheme for animal viruses that is based solely on their structure. The viruses are arranged in order of increasing genome size. It is important to bear in mind that phylogenetic relationships cannot be inferred from this taxonomic scheme. The tables should not be memorized, but instead used as a reference guide to virus structure. In general, viruses with similar structures exhibit similar replication strategies as is discussed in Chapter 6.

TABLE 5–2

**Classification of DNA Animal Viruses**

| Family | Virion Structure | Genome Structure and Molecular Weight | Representative Members |
|---|---|---|---|
| Parvoviruses | Cubic, naked | ss linear ($1–2 \times 10^6$) | Minute virus of mice; adeno-associated viruses |
| Hepatitis B | Cubic, enveloped | ds circular ($2 \times 10^6$), gap in one strand; protein attached | Hepatitis B virus of humans, woodchuck hepatitis virus |
| Papovaviruses | Cubic, naked | ds circular ($3–5 \times 10^6$) | Papillomaviruses, polyomavirus (mouse), SV40 (monkey) |
| Adenoviruses | Cubic, naked | ds linear ($20–25 \times 10^6$); protein attached | Human and animal respiratory disease viruses |
| Herpesviruses | Cubic, enveloped | ds linear ($80–130 \times 10^6$) | Herpes simplex virus types 1 and 2; varicella–zoster virus; cytomegalovirus; Epstein–Barr virus; human herpesvirus 6, human herpesvirus 8 (Kaposi's sarcoma) |
| Poxviruses | Helical, enveloped | ds linear ($160–200 \times 10^6$) | Smallpox; vaccinia; molluscum contagiosum; fibroma and myxoma viruses of rabbits |

*Abbreviations:* ss, single stranded; ds, double stranded.

TABLE 5–3

**Some Important Bacteriophages**

| Bacteriophage | Host | Genome Structure and Molecular Weight | Comments |
|---|---|---|---|
| MS2 | *Escherichia coli* | ss linear RNA ($1.2 \times 10^6$) | Lytic |
| Filamentous (M13, fd) | *Escherichia coli* | ss circular DNA ($2.1 \times 10^6$) | No cell death |
| $\phi$X174 | *Escherichia coli* | ss circular DNA ($1.8 \times 10^6$) | Lytic |
| $\beta$ | *Corynebacterium diphtheriae* | ds linear DNA ($23 \times 10^6$) | Temperate, codes for diphtheria toxin |
| $\lambda$ | *Escherichia coli* | ds linear DNA ($31 \times 10^6$) | Temperate |
| T4 | *Escherichia coli* | ds linear DNA ($108 \times 10^6$) | Lytic |

*Abbreviations:* ss, single stranded; ds, double stranded.

Representative and important bacteriophages are listed along with their properties in Table 5–3. In the chapters to follow the properties of the well-studied temperate bacteriophage, $\lambda$, are described to illustrate the replicative strategies of the more medically important, but less well-studied, $\beta$ phage of *Corynebacterium diphtheriae.*

CHAPTER 6

# Viral Multiplication

JAMES J. CHAMPOUX

A virus multiplication cycle is typically divided into the following discrete phases: (1) adsorption to the host cell, (2) penetration or entry, (3) uncoating to release the genome, (4) virion component production, (5) assembly, and (6) release from the cell. This series of events, sometimes with slight variations, describes what is called the **productive** or **lytic response;** however, this is not the only possible outcome of a virus infection. Some viruses can also enter into a very different kind of relationship with the host cell in which no new virus is produced, the cell survives and divides, and the viral genetic material persists indefinitely in a latent state. This outcome of an infection is referred to as the **nonproductive response.** The nonproductive response is called **lysogeny** in the case of bacteriophages and under some circumstances may be associated with **oncogenic transformation** by animal viruses. (This use of the term transformation is to be distinguished from DNA transformation of bacteria discussed in Chapter 4.)

Viral infections may be productive or nonproductive

Some animal viruses can cause oncogenic transformation

The outcome of an infection depends on the particular virus–host combination and on other factors such as the extracellular environment, multiplicity of infection, and physiology and developmental state of the cell. Those viruses that can enter only into a productive relationship are called **lytic** or **virulent viruses.** Viruses that can establish either a productive or a nonproductive relationship with their host cells are referred to as **temperate viruses.** Some temperate viruses can be reactivated or "induced" to leave the latent state and enter into the productive response. Whether induction occurs depends on the particular virus–host combination, the physiology of the cell, and the presence of extracellular stimuli.

Temperate viruses can either replicate or enter a latent state

The remainder of this chapter is concerned with the details of the steps of the lytic response. In Chapter 7, the topics of lysogeny and oncogenic transformation are considered.

## GROWTH AND ASSAY OF VIRUSES

Viruses are generally propagated in the laboratory by mixing the virus and susceptible cells together and incubating the infected cells until lysis occurs. After lysis, the cells and cell debris are removed by a brief centrifugation and the resulting supernatant is called a **lysate.**

The growth of animal viruses requires that the host cells be cultivated in the laboratory. To prepare cells for growth in vitro, a tissue is removed from an animal and the cells are disaggregated using the proteolytic enzyme trypsin. The cell suspension is seeded into a plastic petri dish in a medium containing a complex mixture of amino acids, vitamins, minerals, and sugars. In addition to these nutritional factors, the growth of animal cells requires components present in animal serum. This method of growing cells is referred to as **tissue culture,** and the initial cell population is called a **primary culture.** The cells attach to the bottom of the plastic dish and remain attached as they divide and eventually cover the surface of the dish. When the culture becomes crowded, the cells generally

Viruses are cultivated in cell cultures derived from animal tissues cease dividing and enter a resting state. Propagation can be continued by removing the cells from the primary culture plate using trypsin and reseeding a new plate.

Permanent cell lines are useful for growing viruses

Cells taken from a normal (as opposed to cancerous) tissue cannot usually be propagated in this manner indefinitely. Eventually most of the cells die; a few may survive, and these survivors often develop into a permanent cell line. Such cell lines are very useful as host cells for isolating and assaying viruses in the laboratory, but they rarely bear much resemblance to the tissue from which they originated. When cells are taken from a tumor and cultivated in vitro, they display a very different set of growth properties, including long-term survival, reflecting their tumor phenotype (see Chapter 7).

Cytopathic effects are characteristic for individual viruses

When a virus is propagated in tissue culture cells, the cellular changes induced by the virus, which usually culminate in cell death, are often characteristic of a particular virus and are referred to as the **cytopathic effect** of the virus (see Chapter 15).

Viruses are quantitated by a plaque assay

Viruses are quantitated by a method called the plaque assay (see Plaque Assay under Quantitation of Viruses for a detailed description of the method). Briefly, viruses are mixed with cells on a petri plate such that each infectious particle gives rise to a zone of lysed or dead cells called a **plaque.** From the number of plaques on the plate, the titer of infectious particles in the lysate is calculated. Virus titers are expressed as the number of plaque-forming units per milliliter (pfu/mL).

## ONE-STEP GROWTH EXPERIMENT

One-step growth experiments are useful in the study of infections

To describe an infection in temporal and quantitative terms it is useful to perform a one-step growth experiment (Fig 6–1). The objective in such an experiment is to infect every cell in a culture so that the whole population proceeds through the infection process in a synchronous fashion. The ratio of infecting plaque-forming units to cells is called the multiplicity of infection (MOI). By infecting at a high MOI (eg, 10 as in Fig 6–1), one can be certain that every cell is infected.

Shortly after infection, a virus loses its identity (eclipse phase)

Infectious virus reappears at end of eclipse phase inside the cell

The time course and efficiency of adsorption can be followed by the loss of infectious virus from the medium after removal of the cells (solid line in Fig 6–1). In the example shown, adsorption takes about a half-hour and all but 1% of the virus is adsorbed. If samples of the culture containing the infected cells are treated so as to break open the cells prior to assaying for virus (broken line in Fig 6–1), it can be observed that infectious virus initially disappears, because no infectious particles are detectable above the background of unadsorbed virus. The period of infection in which no infectious viruses are found inside the cell is called the **eclipse phase** and emphasizes that the original virions lose their infectivity soon after entry. Infectivity is lost because, as is discussed later, the virus particles are dismantled as a prelude to their reproduction. Later, infectious virus

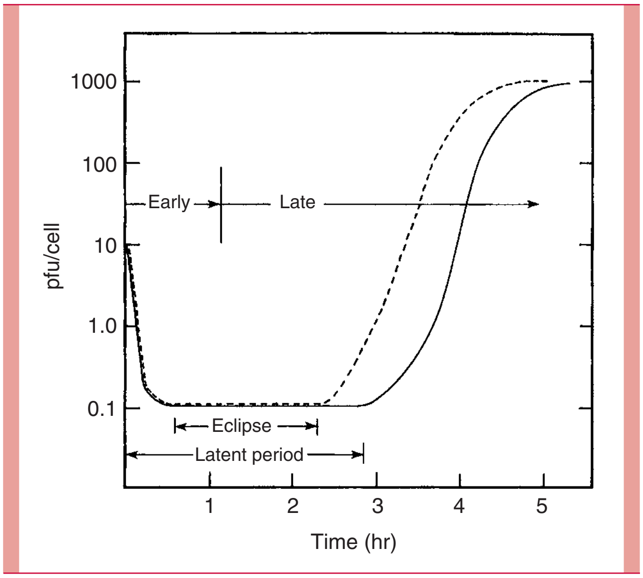


FIGURE 6–1
One-step growth experiment. pfu, plaque-forming units.

particles rapidly reappear in increasing numbers and are detected inside the cell prior to their release into the environment (see Fig 6–1). The length of time from the beginning of infection until progeny virions are found outside the cells is referred to as the **latent period.** Latent periods range from 20 minutes to hours for bacteriophages and from a few hours to many days for animal viruses.

The time in the infection at which genome replication begins is typically used to divide the infection operationally into early and late phases. Early viral gene expression is largely restricted to the production of those proteins required for genome replication; later, the proteins synthesized are primarily those necessary for construction of the new virus particles.

Proteins for replication are produced early and those for construction of virions are produced late

The average number of plaque-forming units released per infected cell is called the burst size for the infection. In the example shown, the burst size is about 1000. Burst sizes range from less than 10 for some relatively inefficient infections to millions for some highly virulent viruses.

## ADSORPTION

The first step in every viral infection is the attachment or adsorption of the infecting particle to the surface of the cell. A prerequisite for this interaction is a collision between the virion and the cell. Viruses do not have any capacity for locomotion, and so the collision event is simply a random process determined by diffusion. Therefore, like any bimolecular reaction, the rate of adsorption is determined by the concentrations of both the virions and the cells.

Only a small fraction of the collisions between a virus and its host cell lead to a successful infection, because adsorption is a highly specific reaction that involves protein molecules on the surface of the virion called **virion attachment proteins** and certain molecules on the surface of the cell called **receptors.** Typically there are $10^4$ to $10^5$ receptors on the cell surface. Receptors for some bacteriophages are found on pili, although the majority adsorb to receptors found on the bacterial cell wall. Receptors for animal viruses are usually glycoproteins located in the plasma membrane of the cell. Table 6–1 lists

Adsorption involves virion attachment proteins and cell surface receptor proteins

TABLE 6–1

### Examples of Viral Receptors

| Virus | Receptor | Cellular Function |
|---|---|---|
| Influenza A | Sialic acid | Glycoprotein |
| Reoviruses | Sialic acid | Glycoprotein |
| | EGF receptor | Signaling |
| Adenoviruses | Integrins | Binding to extracellular matrix |
| Epstein–Barr | CR2 | Complement receptor |
| Herpes simplex | Heparan sulfate | Glycoprotein |
| Human herpes 7 | CD4 | Immunoglobulin superfamily |
| HIV | CD4 | Immunoglobulin superfamily |
| | CXCR4 and CCR5 | Chemokine receptors |
| Human coronavirus | Aminopeptidase N | Protease |
| Human rhinoviruses | ICAM-1 | Immunoglobulin superfamily |
| Measles | CD46 | Complement regulation |
| Poliovirus | PVR | Immunoglobulin superfamily |
| Rabies | Acetylcholine receptor | Signaling |
| SV40 | MHC I | Immunoglobulin superfamily |
| Vaccinia | EGF receptor | Signaling |

*Abbreviations:* EGF, endothelial growth factor; HIV, human immunodeficiency virus; ICAM, intercellular adhesion molecule; MHC, major histocompatibility complex; PVR, poliovirus receptor.

some of the receptors that have been identified for medically important viruses. It appears that viruses have evolved to make use of a wide variety of surface molecules as receptors, which are normally signaling devices or immune system components. Any attempts to design agents that block viral infections by binding to the receptors must consider the possibility that the loss of the normal cellular function associated with the receptors would have serious consequences for the host organism.

For some viruses, two different surface molecules, called **coreceptors,** are involved in adsorption. Although CD4 was originally thought to be the sole receptor for human immunodeficiency virus type 1 (HIV-1), the discovery of a family of coreceptors that normally function as chemokine receptors may explain why natural resistance against the virus is found in individuals with variant forms of these signaling molecules. Receptors for some animal viruses are also found on red blood cells of certain species and are responsible for the phenomena of hemagglutination and hemadsorption discussed later.

Viral spikes and phage tails carry attachment proteins

Virion attachment proteins are often associated with conspicuous features on the surface of the virion. For example, the virion attachment proteins for the bacteriophages with tails are located at the very end of the tails or the tail fibers (Fig 6–2). Likewise, the spikes found on adenoviruses (Fig 5–3E) and on virtually all of the enveloped animal viruses contain the virion attachment proteins.

In some cases, a region of the capsid protein serves the function of the attachment protein. For polioviruses, rhinoviruses, and probably other picornaviruses, the region on the capsid that binds to the receptor is found at the bottom of a cleft or trough that is too narrow to allow access to antibodies. This particular arrangement is clearly advantageous to the virus because it precludes the production of antibodies that might directly block receptor recognition.

Adsorption is enhanced by presence of multiple attachment and receptor proteins

The repeating subunit structure of capsids and the multiplicity of spikes on enveloped viruses are probably important in determining the strength of the binding of the virus to the cell. The binding between a single virion attachment protein and a single receptor protein is relatively weak, but the combination of many such interactions leads to a strong association between the virion and the cell. The fluid nature of the animal cell membrane may facilitate the movement of receptor proteins to allow the clustering that is necessary for these multiple interactions.

A particular kind of virus is capable of infecting only a limited spectrum of cell types called its **host range.** Thus, although a few viruses can infect cells from different species, most viruses are limited to a single species. For example, dogs do not contract measles, and humans do not contract distemper. In many cases, animal viruses infect only a particular subset of the cells found in their host organism. Clearly this kind of tissue tropism is an important determinant of viral pathogenesis. In most cases studied, the specific host range of a virus and its associated tissue tropism are determined at the level of the binding between the cell receptors and virion attachment proteins. Thus, these two protein components must possess complementary surfaces that fit together in much the same way as a substrate fits into the active site of an enzyme. It follows that adsorption occurs only in that fraction of collisions that lead to successful binding between receptors and attachment proteins and that the inability of a virus to infect a cell type is usually due to the absence of the appropriate receptors on the

Differences in host range and tissue tropism are due to presence or absence of receptors

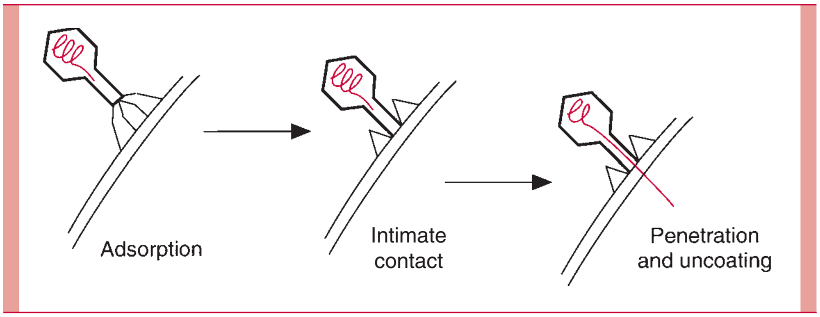


FIGURE 6–2
Bacteriophage entry.

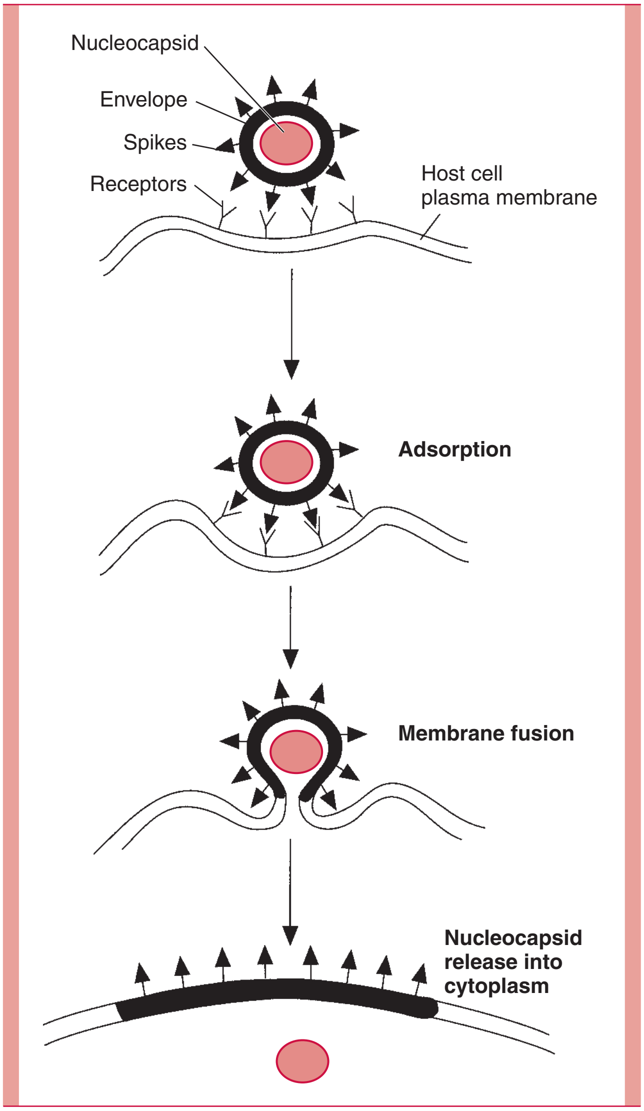


FIGURE 6–3
Entry by direct fusion.

cell. The exquisite specificity of these interactions is well illustrated by the case of a particular mouse reovirus. It has been found that the tissue tropism, and therefore, the resultant pathology, are altered by a point mutation that changes a single amino acid in the virion attachment protein. A few cases are known in which the host range of a virus is determined at a step after adsorption and penetration, but these are the exceptions rather than the rule.

Once a virus particle has penetrated to the inside of a cell, it is essentially hidden from the host immune system. Thus, if protection from a virus infection is to be accomplished at the level of antibody binding to the virions, it must occur before adsorption and prevent the virus from attaching to and penetrating the cell. It is therefore not surprising that most neutralizing antibodies, whether acquired as a result of natural infection or vaccination, are specific for virion attachment proteins.

Neutralizing antibodies are often specific for attachment proteins

## ENTRY AND UNCOATING

The disappearance of infectious virus during the eclipse phase is a direct consequence of the fact that viruses are dismantled prior to being replicated. As is discussed later, the uncoating step may be simultaneous with entry or may occur in a series of steps. Ultimately the nucleocapsid or core structure must be transported to the site or compartment in the cell where transcription and replication will occur.

Viruses are dismantled before being replicated

## The Bacteriophage Strategy

Bacteriophage capsids are shed and only the viral genome enters the host cell

The processes of penetration and uncoating are simultaneous for all bacteriophages. Thus, the viral capsids are shed at the surface, and only the nucleic acid genome enters the cell. In some cases, a small number of virion proteins may accompany the genome into the cell, but these are probably tightly associated with the nucleic acid or are essential enzymes needed to initiate the infection.

Tailed phages attach by tail fibers and DNA is injected through the tail

Bacteriophages with tails have evolved these special appendages to facilitate the entry of the genome into the cell. The process of penetration and uncoating for bacteriophage T4 is shown schematically in Figure 6–2. The tail fibers extending from the end of the tail are responsible for the attachment of the virion to the cell wall, and, in the next step, the end of the tail itself makes intimate contact with the cell surface. Finally the DNA of the virus is injected from the head directly into the cell through the hollow tail structure. The process has been likened to the action of a syringe, but the energetics and the nature of the orifice in the cell surface through which the DNA travels are poorly understood.

## Enveloped Animal Viruses

There are two basic mechanisms for the entry of an enveloped animal virus into the cell. Both mechanisms involve fusion of the viral envelope with a cellular membrane, and the end result in both cases is the release of the free nucleocapsid into the cytoplasm. What distinguishes the two mechanisms is the nature of the cellular membrane that fuses with the viral envelope.

Some enveloped viruses enter cells by direct fusion of plasma membrane and envelope

Paramyxoviruses (eg, measles), some retroviruses (eg, HIV-1), and herpesviruses enter by a process called **direct fusion** (see Fig 6–3). The envelopes of these viruses contain protein spikes that promote fusion of the viral membrane with the plasma membrane of the cell, releasing the nucleocapsid directly into the cytoplasm. Because the viral envelope becomes incorporated into the plasma membrane of the infected cell and still possesses its fusion proteins, infected cells have a tendency to fuse with other uninfected cells. Cell–cell fusion is a hallmark of infections by paramyxoviruses and HIV-1 and can be important in the pathology of diseases such as measles and acquired immunodeficiency syndrome (AIDS).

The mechanism for the entry of most of the remaining enveloped animal viruses, such as orthomyxoviruses (eg, influenza viruses), togaviruses (eg, rubella virus), rhabdoviruses (eg, rabies), and coronaviruses, is shown in Figure 6–4. Following adsorption, the virus particles are taken up by a cellular mechanism called **receptor-mediated endocytosis,** which is normally responsible for internalizing growth factors, hormones, and some nutrients. When it involves viruses, the process is referred to as **viropexis.**

Other enveloped and naked viruses are taken in by receptor-mediated endocytosis (viropexis)

In viropexis, the adsorbed virions become surrounded by the plasma membrane in a reaction that is probably facilitated by the multiplicity of virion attachment proteins on the surface of the particle. Pinching off of the cellular membrane by fusion encloses the virion in a cytoplasmic vesicle termed the **endosomal vesicle.** The nucleocapsid is now surrounded by two membranes, the original viral envelope and the newly acquired endosomal membrane. The surface receptors are subsequently recycled back to the plasma membrane, and the endosomal vesicle is acidified by a normal cellular process. The low pH of the endosome leads to a conformational change in a viral spike protein, which results in the fusion of the two membranes and release of the nucleocapsid into the cytoplasm. In some cases, the contents of the endosomal vesicle may be transferred to a lysosome prior to the fusion step that releases the nucleocapsid.

## Naked Capsid Animal Viruses

Acidified endosome releases nucleocapsid to cytoplasm

Naked capsid viruses, such as poliovirus, reovirus, and adenovirus, also appear to enter the cell by viropexis. However, in this case, the virus cannot escape the endosomal vesicle by membrane fusion as described earlier for enveloped viruses. For poliovirus it appears that the viral capsid proteins in the low-pH environment of the endosome expose hydrophobic domains. This process results in the binding of the virions to the membrane and release of

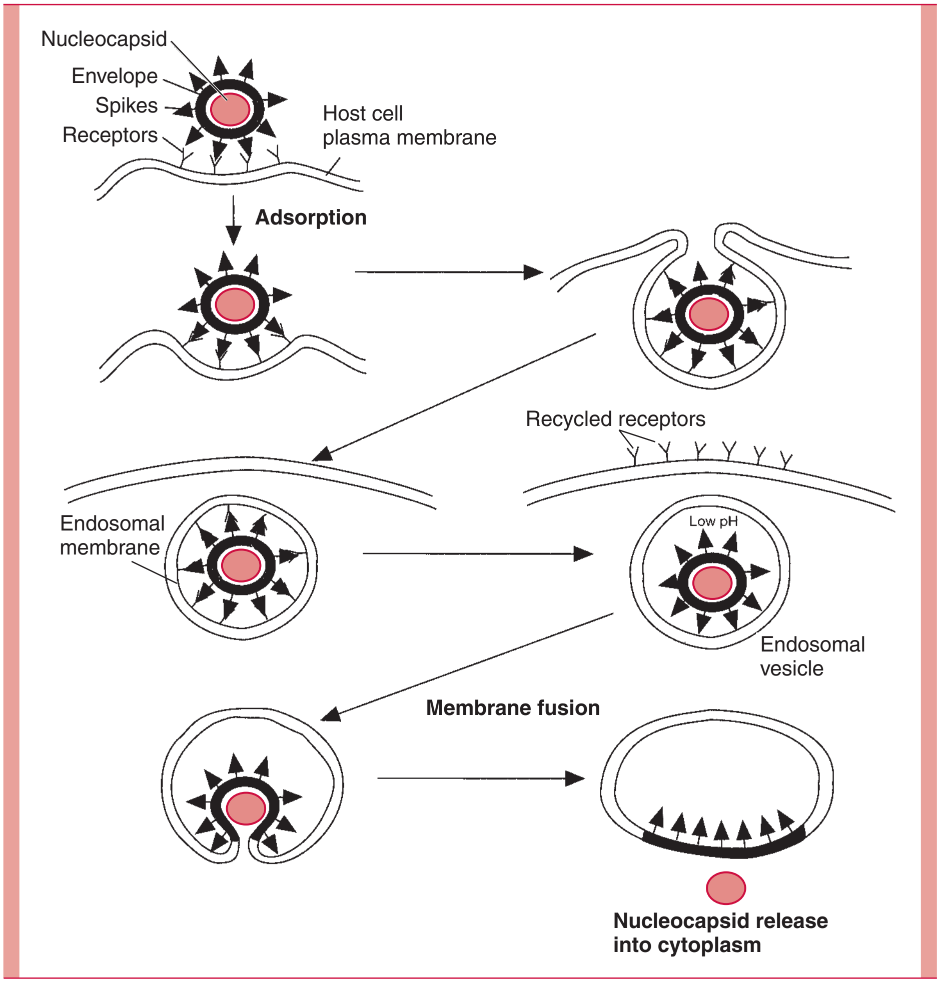


FIGURE 6–4
Viropexis.

the nucleic acid genome into the cytoplasm. In other cases the virions may escape into the cytoplasm by simply promoting the lysis of the vesicle. This step is a potential target of antiviral chemotherapy, and some drugs have been developed that bind to the capsids of picornaviruses and prevent the release of the virus particles from the endosome.

Virions may escape endosome by dissolution of the vesicles

Reovirus is unusual in that prior to release into the cytoplasm, the contents of the endosome are transferred to a lysosome where the lysosomal proteases strip away part of the capsid proteins and activate virion-associated enzymes required for transcription.

## Fate of Intracellular Particles

Even in the relatively simple bacterial cell, there is evidence that the entering nucleic acid must be directed to a particular cellular locus to initiate the infection process. **Pilot proteins** have been described that accompany the phage genome into the bacterial cell and serve the function of "piloting" the nucleic acid to a particular target, such as a membrane site where transcription and replication are to occur.

The ultimate fate of internalized animal virus particles depends on the particular virus and on the cellular compartment where replication occurs. Most RNA viruses with the exception of influenza viruses and the retroviruses replicate in the cytoplasm, the immediate site of entry. Retroviruses, influenza viruses, and all the DNA viruses except the poxviruses must move from the cytoplasm to the nucleus to replicate. The larger DNA viruses, such as herpesviruses and adenoviruses, must uncoat to the level of cores prior to entry into the nucleus. The smaller DNA viruses, such as the parvoviruses and the papovaviruses, enter the nucleus intact through the nuclear pores and subsequently uncoat inside. The largest of the animal viruses, the poxviruses, carry out their entire replicative cycle in the cytoplasm of the infected cell.

Most RNA viruses replicate in cytoplasm

Influenza viruses, retroviruses, and DNA viruses except poxviruses replicate in the nucleus

## THE PROBLEMS OF PRODUCING mRNA

### From Genome to mRNA

Virus-specified mRNAs direct synthesis of viral proteins

An essential step in every virus infection is the production of virus-specific mRNAs that program the cellular ribosomes to synthesize viral proteins. Besides the structural proteins of the virion, viruses must direct the synthesis of enzymes and other specialized proteins required for genome replication, gene expression, and virus assembly and release. The production of the first viral mRNAs at the beginning of the infection is a crucial step in the takeover of the cell by the virus.

Most DNA virus mRNAs are synthesized by host polymerase

(+)-strand RNA virus genome serves as mRNA

For some viruses, the presentation of mRNA to the cellular ribosomes poses no problems. Thus, the genomes of most DNA viruses are transcribed by the host DNA-dependent RNA polymerase to yield the viral mRNAs. The (+)-strand RNA viruses, such as the picornaviruses, the togaviruses, and the coronaviruses, possess genomes that can be used directly as mRNAs and are translated (at least partially, as discussed later) immediately on entry into the cytoplasm of the cell.

Other RNA viruses synthesize and package transcription enzymes to produce initial mRNAs

However, for many viruses, the production of mRNA starting from the genome is not so straightforward. The fact that poxviruses replicate in the cytoplasm means that the cellular RNA polymerase is not available to transcribe the DNA genome. Moreover, no cellular machinery exists that can use either single-stranded or double-stranded RNA as a template to synthesize mRNA. Therefore, the poxviruses and those viruses that utilize an RNA template to make mRNAs must provide their own transcription machinery to produce the viral mRNAs at the beginning of the infection process. This feat is accomplished by synthesizing the transcriptases in the later stages of viral development in the previous host cell and packaging the enzymes into the virions, where they remain associated with the genome as the virus enters the new cell and uncoats. In general, the presence of a transcriptase in virions is indicative that the host cell is unable to use the viral genome as mRNA or as a template to synthesize mRNA. At later times in the infection, any special enzymatic machinery required by the virus and not initially present in the cell, can be supplied among the proteins translated from the first mRNA molecules.

There are a variety of pathways for synthesis of mRNA by different virus groups

The pathways for the synthesis of mRNA by the major virus groups are summarized in Figure 6–5 and related to the structure of viral genomes. The polarity of mRNA is designated as (+) and the polarity of polynucleotide chains complementary to mRNA as (−). The black arrows denote synthetic steps for which host cells provide the required enzymes, whereas the colored arrows indicate synthetic steps that must be carried out by virus-encoded enzymes. Several additional points should be emphasized. The parvoviruses and some phages have single-stranded DNA genomes. Although the RNA polymerase of the cell requires double-stranded DNA as a template, these viruses need not

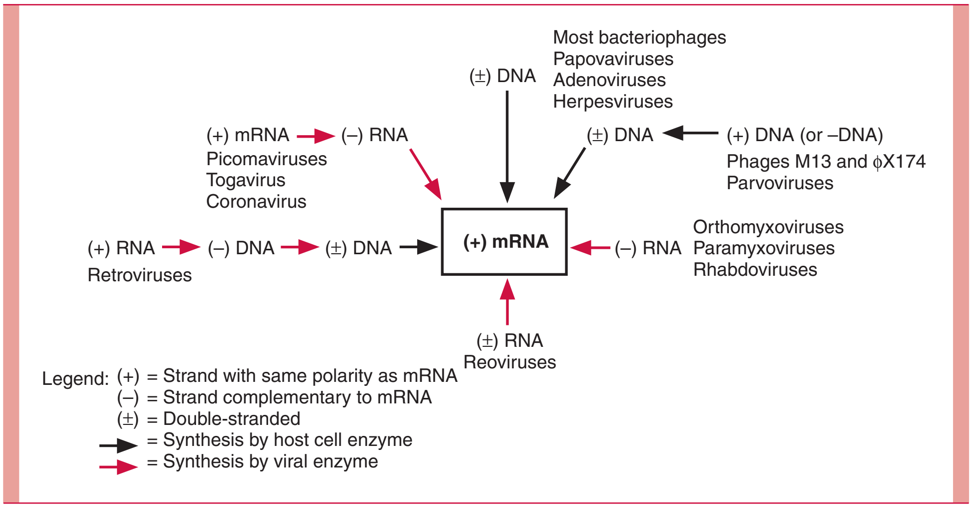


FIGURE 6–5
Pathways of mRNA synthesis for major virus groups.

carry special enzymes in their virions because host cell DNA polymerases can convert the genomes into double-stranded DNA. Note that the production of more mRNA by the picornaviruses and similar (+)-strand RNA viruses requires the synthesis of an intermediate (−)-strand RNA template. The enzyme required for this process is produced by translation of the genome RNA early in infection.

The retroviruses are a special class of (+)-strand RNA viruses. Although their genomes are the same polarity as mRNA and could in principle serve as mRNAs early after infection, their replication scheme apparently precludes this. Instead, the RNA genomes of these viruses are copied into (−)DNA strands by an enzyme carried within the virion called **reverse transcriptase.** The (−)DNA strands are subsequently converted by the same enzyme to double-stranded DNA in a reaction that requires the degradation of the original genomic RNA by the RNase H activity of the reverse transcriptase. The DNA product of reverse transcription is integrated into the host cell DNA and ultimately transcribed by the host RNA polymerase to complete the replication cycle as well as produce viral mRNA. For example, the replication of the hepatitis B DNA genome is mechanistically similar to that of a retrovirus. Thus, the viral DNA is transcribed to produce a single-stranded RNA, which in turn is reverse transcribed to produce the progeny viral DNA that is encapsidated into virions.

Retroviral RNA is copied to DNA by virion reverse transcriptase; host RNA polymerase transcribes DNA into more RNA

## The Monocistronic mRNA Rule in Animal Cells

The ribosome requires input of information in the form of mRNA. For a viral mRNA to be recognized by the ribosome, its production must conform to the rules of structure that govern the synthesis of the cellular mRNAs. Prokaryotic mRNA is relatively simple and can be polycistronic, which means it can contain the information for several proteins. Each cistron or coding region is translated independently beginning from its own ribosome binding site.

Prokaryotic mRNAs can be polycistronic

Eukaryotic mRNAs are structurally more complex, containing special 5′-cap and 3′-poly(A) attachments. In addition, their synthesis often involves removal of internal sequences by a process called **splicing.** Most importantly, virtually all eukaryotic mRNAs are monocistronic. Accordingly, eukaryotic translation is initiated by the binding of a ribosome to the 5′-cap, followed by movement of the ribosome along the DNA until the first AUG initiation codon is encountered. The corollary to this first AUG rule is that eukaryotic ribosomes, unlike prokaryotic ribosomes, generally cannot initiate translation at internal sites on a mRNA. To conform to the monocistronic mRNA, most animal viruses produce mRNAs that are translated to yield only a single polypeptide chain following initiation near the 5′ end of the mRNA.

Animal virus mRNAs are almost always monocistronic

Because most DNA animal viruses replicate in the nucleus, they adhere to the monocistronic mRNA rule either by having a promoter precede each gene or by programming the transcription of precursor RNAs that are processed by nuclear splicing enzymes into monocistronic mRNAs. The virion transcriptase of the cytoplasmic poxviruses apparently must synthesize monocistronic mRNAs by initiation of transcription in front of each gene.

RNA-containing animal viruses have evolved three different strategies to circumvent or conform to the monocistronic mRNA rule. The simplest strategy involves having a segmented genome. For the most part, each genome segment of the orthomyxoviruses and the reoviruses corresponds to a single gene; therefore, the mRNA transcribed from a given segment constitutes a monocistronic mRNA. Unlike most RNA viruses, the orthomyxovirus virus influenza A replicates in the nucleus, and some of its monocistronic mRNAs are produced by splicing of precursor RNAs by host cell enzymes. Moreover, orthomyxoviruses use small 5′ RNA fragments derived from host cell pre-mRNAs found in the nucleus to prime the synthesis of their own mRNAs.

Some RNA viruses have segmented genomes

A second solution to the monocistronic mRNA rule is very similar to the strategy employed by cells and the DNA viruses. The paramyxoviruses, togaviruses, rhabdoviruses, filoviruses, bunyaviruses, arenaviruses, and coronaviruses synthesize monocistronic mRNAs by initiating the synthesis of each mRNA at the beginning of a gene. In most cases, the transcriptase terminates mRNA synthesis at the end of the gene so that each message corresponds to a single gene. For coronaviruses, RNA synthesis initiates at the beginning of each

Some viruses make monocistronic RNAs by initiating synthesis at the start of each gene

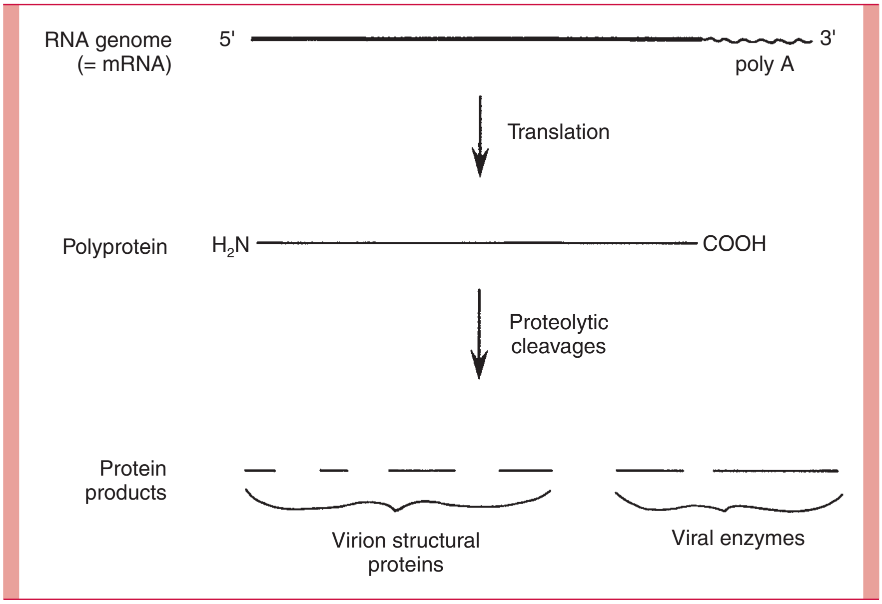


FIGURE 6–6
Poliovirus gene expression.

gene and continues to the end of the genome so that a nested set of mRNAs is produced. However, each mRNA is functionally monocistronic and is translated to produce only the protein encoded near its 5′ end.

Picornaviruses make a polyprotein that is proteolytically cleaved later

The picornaviruses have evolved yet a third strategy to deal with the monocistronic mRNA requirement (Fig 6–6). The (+)-strand genome contains just a single ribosome binding site near the 5′ end. It is translated into one long polypeptide chain called a **polyprotein,** which is subsequently broken into the final set of protein products by a series of proteolytic cleavages. Most of the required protease activities reside within the polyprotein itself.

Several viruses use more than one of these strategies to conform to the monocistronic mRNA rule. For example, retroviruses, togaviruses, arenaviruses, and bunyaviruses synthesize multiple mRNAs, each one coding for a polyprotein that is subsequently cleaved into the individual protein molecules.

## GENOME REPLICATION

### DNA Viruses

Cells obviously contain the enzymes and accessory proteins required for the replication of DNA. In bacteria these proteins are present continuously, whereas in the eukaryotic cell they are present only during the S phase of the cell cycle, and they are restricted to the nucleus. The extent to which viruses use the cell replication machinery depends on their protein-coding potential and thus on the size of their genome.

The smallest DNA viruses depend exclusively on host DNA replication machinery

The largest DNA viruses code for enzymes important for DNA replication

The smallest of the DNA viruses, the parvoviruses, are so completely dependent on host machinery that they require the infected cells to be dividing so that a normal S phase will occur and replicate the viral DNA along with the cellular DNA. At the other end of the spectrum are the large DNA viruses, which are relatively independent of cellular functions. The largest bacteriophages such as T4 degrade the host cell chromosome early in infection and replace all of the host replication machinery with virus-specified proteins. The largest animal viruses, the poxviruses, are similarly independent of the host. Because they replicate in the cytoplasm, they must code for virtually all of the enzymes and other proteins required for replicating their DNA.

The remainder of the DNA viruses are only partially dependent on host machinery. For example, bacteriophages $\phi$X174 and $\lambda$ code for proteins that direct the initiation of DNA synthesis to the viral origin. However, the actual synthesis of DNA occurs by the complex of cellular enzymes responsible for replication of the *Escherichia coli* DNA. Similarly the small DNA animal viruses, such as the papovaviruses, code for a protein that is involved in the initiation of synthesis at the origin, but the remainder of the replication process is carried out by host machinery. The somewhat more complex adenoviruses and herpesviruses, in addition to providing origin-specific proteins, also code for their own DNA polymerases and other accessory proteins required for DNA replication.

Herpesvirus-encoded DNA polymerase is a target of chemotherapy (eg, acyclovir)

The fact that the herpesviruses code for their own DNA polymerase has important implications for the treatment of infections by these viruses and illustrates a central principle of antiviral chemotherapy. Certain antiviral drugs (adenine arabinoside and 5′-iododeoxyuridine) have been found to be effective against herpesvirus infections (see Chapter 38); they are sufficiently similar to natural substrates that the virally encoded DNA polymerase mistakenly incorporates them into viral DNA, resulting in an inhibition of subsequent DNA synthesis. The host cell enzyme is more discriminating and fails to use the analogs in the synthesis of cellular DNA; thus, the drugs do not kill uninfected cells. The same principle applies to the chain-terminating drugs such as zidovudine (AZT) and dideoxyinosine (ddI) that target the HIV-1 reverse transcriptase. Similarly, the antiviral drugs acyclovir (acycloguanosine) and ganciclovir preferentially kill herpesvirus-infected cells because the viral nucleoside kinases, unlike the cellular counterparts, phosphorylate the nucleoside analog, converting it to a form that inhibits further DNA synthesis when DNA polymerases incorporate it into DNA. In principle, any viral process that is distinct from a normal cellular process is a potential target for antiviral drugs. As more becomes known about the details of viral replication, more drugs will become available that are targeted to these unique viral processes.

Viral processes that are distinct from normal cellular processes are potential targets for antiviral drugs

As noted earlier, with the exception of the poxviruses, all of the DNA animal viruses are at least partially dependent on host cell machinery for the replication of their genomes. However, unlike the parvoviruses, the other DNA viruses do not need to infect dividing cells for a productive infection to ensue. Instead, all of these viruses code for a protein expressed early in infection that induces an unscheduled cycle of cellular DNA replication (S phase). In this way, these viruses ensure that the infected cell makes all of the machinery required for the replication of their own DNA. It is noteworthy that all of the DNA viruses except the parvoviruses are capable, in some circumstances, of transforming a normal cell into a cancer cell (see Chapter 7). This correlation suggests that the unlimited proliferative capacity of the cancer cells may be due to the continual synthesis of the viral protein(s) responsible for inducing the unscheduled S phase in a normal infection. The fact that these DNA viruses can induce oncogenic transformation of cell types that are nonpermissive for viral multiplication may simply be an accident related to the need to induce cellular enzymes required for DNA replication during the lytic infection.

All DNA viruses except parvoviruses can transform host cells

All DNA polymerases, including those encoded by viruses, synthesize DNA chains by the successive addition of nucleotides onto the 3′ end of the new DNA strand. Moreover, all DNA polymerases require a primer terminus containing a free 3′-hydroxyl to initiate the synthesis of a DNA chain. In cellular replication, a temporary primer is provided in the form of a short RNA molecule. This primer RNA is synthesized by an RNA polymerase, and after elongation by the DNA polymerase it is removed. With circular chromosomes, such as those found in bacteria and many viruses, the unidirectional chain growth and primer requirement of the DNA polymerase pose no structural problems for replication. However, as illustrated in Figure 6–7, when a replication fork encounters the end of a linear DNA molecule, one of the new chains (heavy lines) cannot be completed at its 5′ end, because there exists no means of starting the DNA portion of the chain exactly at the end of the template DNA. Thus, after the RNA primer is removed, the new chain is incomplete at its 5′ end. This constraint on the completion of DNA chains on a linear template is called the **end problem** in DNA replication. Some eukaryotic cells add short repetitive sequences to chromosome ends using an enzyme called telomerase to prevent the shortening of the DNA with each successive round of replication.

Replication of linear viral DNAs must solve the end problem

Several viruses are faced with the end problem during replication of their linear genomes, but none use the cellular telomerase to synthesize DNA ends. It is beyond the scope of this text to detail all of the strategies viruses have evolved to deal with the end problem, but it is worth mentioning some of the structural features found in linear viral genomes whose presence is related to solutions of the end problem. These structures are diagrammed schematically in Figure 6–8. The linear double-stranded genome of

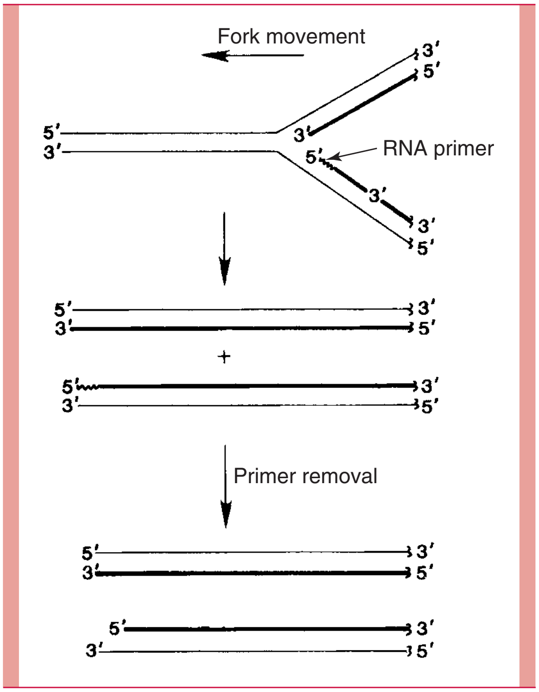


FIGURE 6–7
The end problem in DNA replication.

bacteriophage λ possesses 12-bp single-stranded extensions that are complementary in sequence to each other and thus called **cohesive ends.** Very early after entry into the cell, the two ends pair up to convert the linear genome into a circular molecule to avoid the end problem in replication. The linear double-stranded adenovirus genome contains a protein molecule covalently attached to the 5′ end of both strands. These proteins provide the primers required to initiate the synthesis of the DNA chains during replication, circumventing the need for RNA primers and thus solving the end problem in replication. The single-stranded parvovirus genome contains a self-complementary sequence at the 3′ end that causes the molecule to fold into a hairpin, making it self-priming for DNA replication. The poxviruses contain linear double-stranded genomes in which the ends are continuous. With the parvovirus and poxvirus genomes, the solutions to the end problem create additional problems that must be solved to produce replication products that are identical to the starting genomes.

## RNA Viruses

RNA viruses must encode their own transcriptases

Because nuclear functions are primarily designed for DNA metabolism, RNA animal viruses generally replicate in the cytoplasm. Moreover, cells do not have RNA polymerases that can copy RNA templates. Therefore, RNA viruses not only need to code for transcriptases, as discussed earlier, but also must provide the replicases required to duplicate the genome RNA. Furthermore, except in the cases of the RNA phage and the

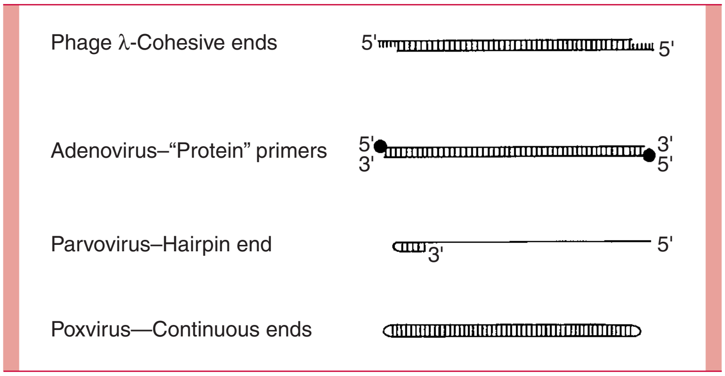


FIGURE 6–8
Some solutions to the end problem.

picornaviruses, where transcription and replication are synonymous, the RNA viruses must temporally and functionally separate replication from transcription. This requirement is especially apparent for the rhabdoviruses, paramyxoviruses, togaviruses, and coronaviruses, where a complete genome, or complementary copy of the genome, is transcribed into a set of small monocistronic mRNAs early in infection. After replication begins, these same templates are used to synthesize full-length strands for replication.

Two mechanisms exist to separate the process of replication from transcription. First, in some cases, transcription is restricted to subviral particles and involves a transcriptase transported into the cell within the virion. Second, in other cases, the replication process involves either a functionally distinct RNA polymerase or depends on the presence of some other viral-specific accessory protein that directs the synthesis of full-length copies of the template rather than the shorter monocistronic mRNAs. In the case of the reoviruses, the switch from transcription to replication appears to involve the synthesis of a replicase that converts the (+)mRNAs synthesized early in infection to the double-stranded genome segments.

Transcription and replication must be separated for most RNA viruses

Viral RNA polymerases, like DNA polymerases, synthesize chains in only one direction; however, in general, RNA polymerases can initiate the synthesis of new chains without primers. Thus, there is no obvious end problem in RNA replication. There is one exception to this general rule. The picornaviruses contain a protein that is covalently attached to the 5′ end of the genome, called Vpg. This protein is present on the viral RNA because it is involved in the priming of new RNA viral genomes during the infection, similar to the process described earlier for adenoviruses.

Picornaviruses use a protein to prime RNA synthesis

## ASSEMBLY OF NAKED CAPSID VIRUSES AND NUCLEOCAPSIDS

The process of enclosing the viral genome in a protein capsid is called assembly or **encapsidation.** Four general principles govern the construction of capsids and nucleocapsids. First, the process generally involves self-assembly of the component parts. Second, assembly is stepwise and ordered. Third, individual protein structural subunits or protomers are usually preformed into capsomeres in preparation for the final assembly process. Fourth, assembly often initiates at a particular locus on the genome called a **packaging site.**

Capsids and nucleocapsids self-assemble from preformed capsomeres

### Viruses With Helical Symmetry

The assembly of the cylindrically shaped tobacco mosaic virus (TMV) has been extensively studied and provides a model for the construction of helical capsids and nucleocapsids. For TMV, doughnut-shaped disks containing a number of individual structural subunits are preformed and added stepwise to the growing structure. Elongation occurs in both directions from a specific packaging site on the single-stranded viral RNA (Fig 6–9). The addition of

Tobacco mosaic virus is a model for the construction of viral components

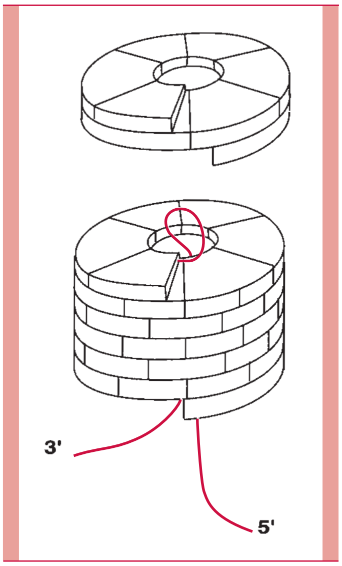


FIGURE 6–9
Tobacco mosaic virus assembly.

each disk involves an interaction between the protein subunits of the disk and the genome RNA. The nature of this interaction is such that the assembly process ceases when the ends of the RNA are reached. The structural subunits as well as the RNA trace out a helical path in the final virus particle.

The basic design features worked out for TMV probably apply in general to the assembly of the nucleocapsids of enveloped viruses. Thus, it is likely that the individual protein subunits are intimately associated with the RNA and that the nucleoprotein complexes are assembled by the stepwise addition of protein subunits or complexes of subunits. For influenza and the other helical viruses with segmented genomes, the various genome segments are assembled into nucleocapsids independently and then brought together during virion assembly by a mechanism that is as yet poorly understood. It is notable that virtually all of the animal RNA viruses with helical symmetry are enveloped.

## Viruses with Cubic Symmetry

Icosahedral capsids are generally preassembled, and the genomes are threaded in

For both phage and animal viruses, icosahedral capsids are generally preassembled and the nucleic acid genomes, usually complexed with condensing proteins, are threaded into the empty structures. Construction of the hollow capsids appears to occur by a self-assembly process, sometimes aided by other proteins. The stepwise assembly of components involves the initial aggregation of structural subunits into pentamers and hexamers, followed by the condensation of these capsomeres to form the empty capsid. In some cases, it appears that a small complex of capsid proteins associates specifically with the viral genome and nucleates the assembly of the complete capsid around the genome.

Phage heads, tails, and tail fibers are synthesized separately and then assembled

The morphogenesis of a complex bacteriophage such as T4 involves the prefabrication of each of the major substructures by a separate pathway, followed by the ordered and sequential construction of the final particle from its component parts (Fig 6–10). An intermediate in the assembly of a bacteriophage head is an empty structure containing an internal protein network that is removed prior to insertion of the nucleic acid. The constituents of this network are often appropriately referred to as **scaffolding proteins,** which apparently provide the lattice necessary to hold the capsomeres in position during the early stages of head assembly.

Some phage DNA is replicated to produce concatemers

For many DNA bacteriophages and the herpesviruses, the products of replication are long linear DNA molecules called **concatemers,** which are made up of tandem head-to-tail repeats of genome-size units. During the threading of the DNA into the preformed capsids, these concatemers are cleaved by virus-encoded nucleases to generate genome-size pieces.

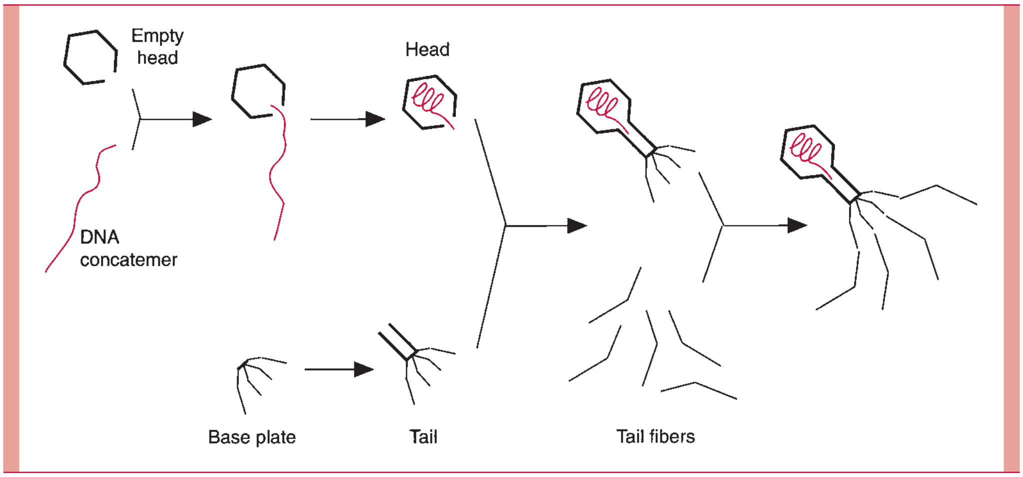


FIGURE 6–10
Assembly of bacteriophage T4.

There are two mechanisms for determining the correct sites for nuclease cleavage during packaging of a concatemer. Bacteriophage λ and the herpesviruses typify one type of mechanism in which the enzyme that makes the cuts is a sequence-specific nuclease. The enzyme sits poised at the orifice of the capsid as the DNA is being threaded into the capsid, and just before the specific cut site enters, the DNA is cleaved. For bacteriophage λ, the breaks are made in opposite strands, 12 bp apart, to generate the cohesive ends. Bacteriophages T4 and P1 are examples of bacterial viruses that illustrate the second mechanism. For these phages, the nuclease does not recognize a particular DNA sequence, but instead cuts the concatemer when the capsid is full. Because the head of the bacteriophage can accommodate slightly more than one genome equivalent of DNA and packaging can begin anywhere on the DNA, the "headful" mechanism produces genomes that are terminally redundant (the same sequence is found at both ends) and circularly permuted. The nonspecific packaging with respect to DNA sequence explains why bacteriophage P1 is capable of incorporating host DNA into phage particles, thereby promoting generalized transduction (see Chapter 4). Bacteriophage T4 does not carry out generalized transduction, because the bacterial DNA is completely degraded to nucleotides early in infection.

Mechanisms for cutting phage DNA during packaging involve site-specific nucleases or headful cleavage

Host DNA may be incorporated by the headful mechanism, and generalized transduction results

## RELEASE

### Bacteriophages

Most bacteriophages escape from the infected cell by coding for one or more enzymes synthesized late in the latent phase that causes the lysis of the cell. The enzymes are either lysozymes or peptidases that weaken the cell wall by cleaving specific bonds in the peptidoglycan layer. The damaged cells burst as a result of osmotic pressure.

Phages encode lysozyme or peptidases that lyse bacterial cell walls

### Animal Viruses

#### *CELL DEATH*

Nearly all productively infected cells die (see below for exceptions), presumably because the viral genetic program is dominant and precludes the continuation of normal cell functions required for survival. In many cases, direct viral interference with normal cellular metabolic processes leads to cell death. For example, picornaviruses shut off host protein synthesis soon after infection, and many DNA animal viruses interfere with normal cell cycle controls. In many cases, the end result of such insults is a triggering of a cellular stress response called programmed cell death or **apoptosis.** Some viruses are known to code for proteins that block or delay apoptosis, probably to stave off cell death until the virus replication cycle has been completed. Ultimately, the cell lysis that accompanies cell death is responsible for the release of naked capsid viruses into the environment.

Naked capsid viruses lacking specific lysis mechanisms are released with cell death

Some viruses block or delay apoptosis to allow completion of the virus replication cycle

#### *BUDDING*

With the exception of the poxviruses, all enveloped animal viruses acquire their membrane by budding either through the plasma membrane or, in the case of herpesviruses, through the membrane of an exocytic vesicle. Thus, for these viruses, release from the cell is coupled to the final stage of virion assembly. How the herpesviruses ultimately escape from the cell when the membrane of the exocytic vesicle fuses with the plasma membrane. The poxviruses appear to program the synthesis of their own outer membrane. How the poxvirus envelope is assembled on the nucleocapsid is not known.

Most enveloped viruses acquire an envelope during release by budding

Poxviruses synthesize their own envelopes

The membrane changes that accompany budding appear to be just the reverse of the entry process described before for those viruses that enter by direct fusion (compare Fig 6–3 and Fig 6–11). The region of the cellular membrane where budding is to occur

The membrane site for budding first acquires virus-specified spikes and matrix protein

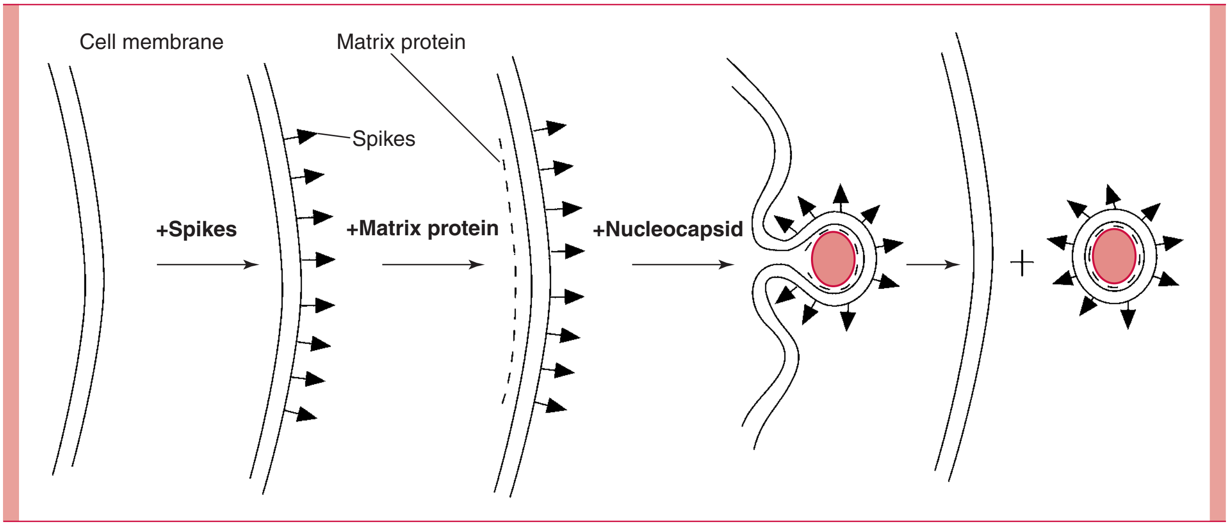


FIGURE 6–11
Viral release by budding.

acquires a cluster of viral glycoprotein spikes. These proteins are synthesized by the pathway that normally delivers cellular membrane proteins to the surface of the cell by way of the Golgi apparatus. At the site of the glycoprotein cluster, the inside of the membrane becomes coated with a virion structural protein called the **matrix** or **M protein.** The accumulation of the matrix protein at the proper location is probably facilitated by the presence of a binding site for the matrix protein on the cytoplasmic side of the transmembrane glycoprotein spike. The matrix protein attracts the completed nucleocapsid that triggers the envelopment process leading to the release of the completed particle to the outside (see Fig 6–11).

For viruses that bud, it is important to note that the plasma membrane of the infected cell contains virus-specific glycoproteins that represent foreign antigens. This means that infected cells become targets for the immune system. In fact, cytotoxic T lymphocytes that recognize these antigens can be a significant factor in combating a virus infection.

The budding process rarely causes cell death

Most retroviruses (except HIV) reproduce without cell death

The process of viral budding usually does not lead directly to cell death because the plasma membrane can be repaired following budding. It is likely that cell death for most enveloped viruses, as for naked capsid viruses, is related to the loss of normal cellular functions required for survival or as a result of apoptosis. Unlike most retroviruses that do not kill the host cell, HIV-1 is cytotoxic. Although the mechanism of HIV-1 cell killing is not entirely understood, factors such as the accumulation of viral DNA in the cytoplasm, the toxic effects of certain viral proteins, alterations in plasma membrane permeability, and cell–cell fusion, are believed to contribute to the cytotoxic potential of the virus.

## *CELL SURVIVAL*

Filamentous phages assemble during extrusion without damaging cells

For retroviruses (except HIV-1 and other lentiviruses) and the filamentous bacteriophages, virus reproduction and cell survival are compatible. Retroviruses convert their RNA genome into double-stranded DNA, which integrates into a host cell chromosome and is transcribed just like any other cellular gene (see Chapter 42). Thus, the impact on cellular metabolism is minimal. Moreover, the virus buds through the plasma membrane without any permanent damage to the cell.

Because the filamentous phages are naked capsid viruses, cell survival is even more remarkable. In this case, the helical capsid is assembled onto the condensed single-stranded DNA genome as the structure is being extruded through both the membrane and the cell wall of the bacterium. How the cell escapes permanent damage in this case is unknown. As with the retroviruses, the infected cell continues to produce virus indefinitely.

## QUANTITATION OF VIRUSES

### Hemagglutination Assay

For some animal viruses, red blood cells from one or more animal species contain receptors for the virion attachment proteins. Because the receptors and attachment proteins are present in multiple copies on the cells and virions, respectively, an excess of virus particles coats the cells and causes them to aggregate. This aggregation phenomenon was first discovered with influenza virus and is called **hemagglutination.** The virion attachment protein on the influenza virion is appropriately called the **hemagglutinin.** Furthermore, the presence of the hemagglutinin in the plasma membrane of the infected cell means that the cells as well as the virions will bind the red blood cells. This reaction, called **hemadsorption,** is a useful indicator of infection by certain viruses (see Chapter 15).

Virion and infected cell attachment proteins also bind red blood cells

Hemagglutination can be used to estimate the titer of virus particles in a virus-containing sample. Serially diluted samples of the virus preparation are mixed with a constant amount of red blood cells, and the mixture is allowed to settle in a test tube. Agglutinated red blood cells settle to the bottom to form a thin, dispersed layer. If there is insufficient virus to agglutinate the red blood cells, they will settle to the bottom of the tube and form a tight pellet. The difference is easily scored visually and the endpoint of the agglutination is used as a relative measure of the virus concentration in the sample.

### Plaque Assay

The plaque assay is a method for determining the titer of infectious virions in a virus preparation or lysate. The sample is diluted serially and an aliquot of each dilution is added to a vast excess of susceptible host cells. For an animal virus, the host cells are usually attached to the bottom of a plastic petri dish; for bacterial cells, adsorption is typically carried out in a cell suspension. In both cases the cells are then immersed in a semisolid medium such as agar, which prevents the released virions from spreading throughout the entire cell population. Thus the virus released from the initial and subsequent rounds of infection can only invade the cells in the immediate vicinity of the initial infected cell on the plate. The end result is an easily visible clearing of dead cells at each of the sites on the plate where one of the original infected cells was located. The clearing is called a **plaque** (Fig 6–12). Visualization

Plaque assay: dilutions of virus are added to excess cells immobilized in agar

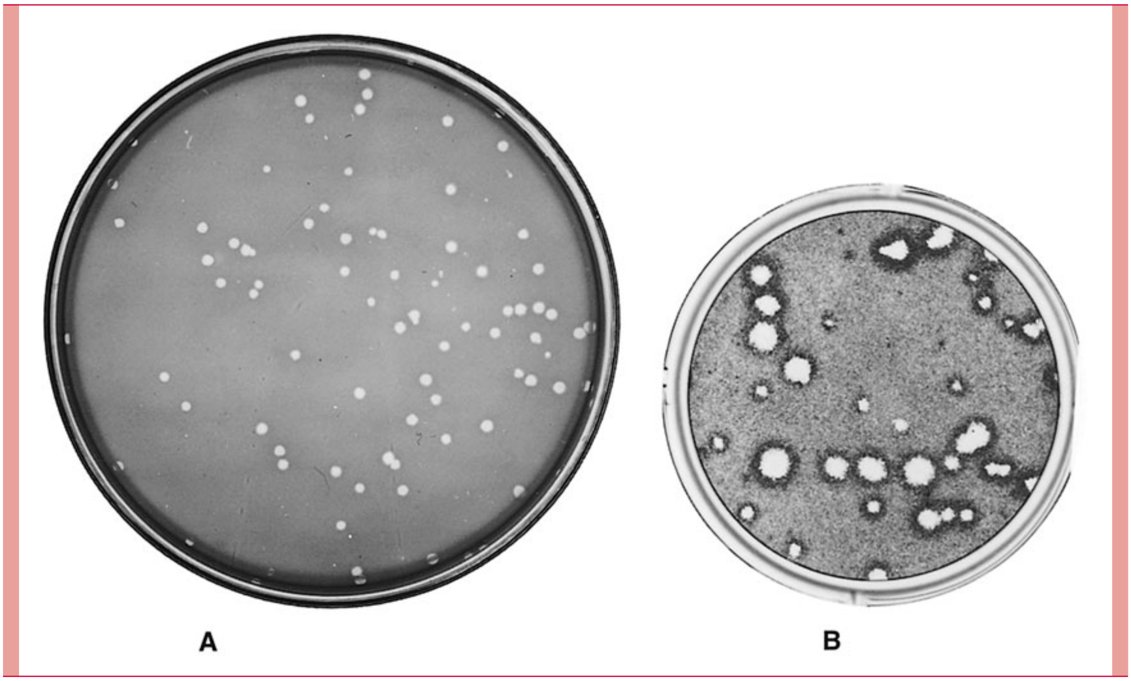


FIGURE 6–12
Plaque assays: **A.** Bacteriophage $\lambda$. **B.** Adenovirus.

Replicated virus infects only neighboring cells, producing countable plaques

in the case of animal cells usually requires staining the cells. By counting the number of plaques and correcting for the dilution factor, the virus titer in the original sample can be calculated. The titer is usually expressed as the number of plaque-forming units per milliliter (pfu/mL).

## INTERFERONS

Interferons are chemokines produced by virally infected cells that inhibit virus production in other cells

Interferons are not virus specific

Interferons are host-encoded proteins that provide the first line of defense against viral infections. They belong to the class of molecules called **chemokines,** which are proteins or glycoproteins that are involved in cell-to-cell communication. Virus infection of all types of cells stimulates the production and secretion of either interferon $\alpha$ or interferon $\beta$, which acts on other cells to induce what is called the **antiviral state.** Unlike immunity, the interferons are not specific to a particular kind of virus; however, interferons usually act only on cells of the same species. Other agents stimulate the production of interferon $\gamma$ by lymphoid cells. In this case, interferon appears to play an important role in the immune system independent of any role as an antiviral protein (see Chapter 10).

Interferons are produced in response to accumulation of double-stranded RNA during viral synthesis

The signal that leads to the production of interferon by an infected cell appears to be double-stranded RNA. This conclusion is based on the observation that treatment of cells with purified double-stranded RNA or synthetic double-stranded ribopolymers results in the secretion of interferon. Although the mechanisms are largely unclear and probably vary from one virus to another, viral infections in general lead to the accumulation of significant levels of double-stranded RNA in the cell.

Interferons inhibit viral protein synthesis by inducing cellular enzymes that require double-stranded RNA

All protein synthesis is inhibited but only in infected cells

Changes in the synthesis of a large number of cellular proteins are characteristic of the antiviral state induced by interferon. However, the cells exhibit only minimal changes in their metabolic or growth properties. The machinery to inhibit virus production is mobilized only on infection. Interferon has multiple effects on cells, but only three systems have been extensively studied. The first system involves a protein called Mx that is induced by interferon and specifically blocks influenza infections by interfering with viral transcription. The second system involves the upregulation of protein kinase that is dependent on double-stranded RNA and PKR which phosphorylates and thereby inactivates one of the subunits of an initiation factor (eIF-2) necessary for protein synthesis. In some cases, viruses have evolved quite specific mechanisms to block the action of this protein kinase. The third system involves the induction of an enzyme called 2′,5′-oligoadenylate synthetase, which synthesizes chains of 2′,5′-oligo(A) up to 10 residues in length. In turn, the 2′,5′-oligo(A) activates a constitutive ribonuclease, called RNase L, that degrades mRNA. The activities of both PKR and 2′,5′-oligo(A) synthetase require the presence of double-stranded RNA, the intracellular signal that an infection is occurring. This requirement prevents interferon from having an adverse effect on protein synthesis in uninfected cells. In these latter two cases, viral infection of a cell that has been exposed to interferon results in a general inhibition of protein synthesis, leading to cell death and no virus production. A cell that was destined to die anyway from a viral infection is sacrificed for the benefit of the entire organism.

## ADDITIONAL READING

Knipe DM, Howley PM, eds. *Fields Virology.* 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. A current and comprehensive overview of animal viruses.

White DO, Fenner FJ, eds. *Medical Virology*. 4th ed. San Diego: Academic Press; 1994. A good overview of medical aspects of virology.

CHAPTER 7

# Viral Genetics

JAMES J. CHAMPOUX

In the typical lytic infection described in Chapter 6, viruses invade a host cell and usurp the machinery of the cell for their own reproduction. The end result is usually cell death with the release of large numbers of new infectious virus particles, most of which are phenotypically identical to the original invading virus. This apparent homogeneity is deceptive.

This chapter considers the methods whereby viral genomes change by mutation and recombination and examines the medical consequences of some of these changes. In addition, it also discusses the methods used by temperate viruses to enter, maintain, and sometimes leave the latent state. Furthermore, it examines in some detail the means by which both bacterial and animal cells can be permanently changed by viral latency.

## MECHANISMS OF GENETIC CHANGE

For DNA bacteriophages, the ratio of infectious particles to total particles usually approaches a value of one. Such is not the case for animal viruses. Typically fewer than 1% of the particles derived from a cell infected with an animal virus are infectious in other cells as determined by a plaque assay. Although some of this discrepancy may be attributable to inefficiencies in the assay procedures, it is clear that many defective particles are being produced. In part, this production of defective particles arises because the mutation rates for animal viruses are unusually high and because many infections occur at high multiplicities, where defective genomes are complemented by nondefective viruses and therefore propagated.

Most of the animal virus particles from an infected cell are defective

### Mutation

Many DNA viruses use the host DNA synthesis machinery for replicating their genomes. Therefore, they benefit from the built-in proofreading and other error-correcting mechanisms used by the cell. However, the largest animal viruses (adenoviruses, herpesviruses, and poxviruses) code for their own DNA polymerases, and these enzymes are not as effective at proofreading as the cellular polymerases. The resulting higher error rates in DNA replication endow the viruses with the potential for a high rate of evolution, but they are also partially responsible for the high frequency of defective viral particles.

The replication of RNA viruses is characterized by even higher error rates because viral RNA polymerases do not possess any proofreading capabilities. The result is that error rates for RNA viruses commonly approach one mistake for every 2500 to 10,000 nucleotides polymerized. Such a high misincorporation rate means that even for the smallest RNA viruses, virtually every round of replication introduces one or more nucleotide changes somewhere in the genome. If it is assumed that errors are introduced at random, most of the members of a clone (eg, in a plaque) are genetically different from all other members of the clone. The resulting mixture of different genome sequences for a particular RNA virus has been referred to as a **quasispecies** to emphasize that the level of genetic variation is much greater than what normally exists in a species.

High error rates for RNA viruses produce genetically heterogeneous populations

Because of the redundancy in the genetic code, some mutations are silent and are not reflected in changes at the protein level, but many occur in essential genes and contribute to the large number of defective particles found for RNA animal viruses. The concept of genetic stability takes on a new meaning in view of these considerations, and the RNA virus population as a whole maintains some degree of homogeneity only because of the high degree of fitness exhibited by a subset of the possible genome sequences. Thus, strong selective forces continually operate on a population to eliminate most mutants that fail to compete with the few very successful members of the population. However, any time the environment changes (eg, appearance of neutralizing antibodies), a new subset of the population is selected and maintained as long as the selective forces remain constant.

High mutation rates permit adaptation to changed conditions

Mutations are responsible for antigenic drift in influenza viruses

The high mutation rates found for RNA viruses endow them with a genetic plasticity that leads readily to the occurrence of genetic variants and permits rapid adaptation to new environmental conditions. The large number of serotypes of the rhinoviruses causing the common cold, for instance, likely reflect the potential to vary by mutation. Although rapid genetic change occurs for most if not all viruses, no medically important RNA virus has exhibited this phenomenon as conspicuously as influenza virus. Point mutations accumulate in the influenza genes coding for the two envelope proteins (hemagglutinin and neuraminidase), resulting in changes in the antigenic structure of the virions. These changes lead to new variants not recognized by the immune system of previously infected individuals. This phenomenon is called **antigenic drift** (see Chapter 33). Apparently, those domains of the two envelope proteins that are most important for immune recognition are not essential for virus reproduction and, as a result, can tolerate amino acid changes leading to antigenic variation. This feature may distinguish influenza from other human RNA viruses that possess the same high mutation rates, but do not exhibit such high rates of antigenic drift. Antigenic drift in epidemic influenza viruses from year to year requires continual updating of the strains used to produce immunizing vaccines.

Retroviruses use two error-prone polymerases for replication

The retroviruses likewise show high rates of variation because they depend for their replication on two different polymerases, both of which are error prone. In the first step of the replication cycle, the reverse transcriptase that copies the RNA genome into double-stranded DNA lacks a proofreading capability. Once the viral DNA has integrated into the chromosome of the host cell, the DNA is transcribed by the host RNA polymerase II, which similarly is incapable of proofreading. Accordingly, the retroviruses, including human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS), exhibit a high rate of mutation. This property gives them the ability to evolve rapidly in response to changing conditions in the infected host.

HIV-1 antigenic variation makes vaccine development difficult

Retroviruses that exhibit high rates of antigenic variation such as HIV-1 pose particularly difficult problems for the development of effective vaccines. Attempts are being made to identify conserved, and therefore presumably essential, domains of the envelope proteins for these viruses, which might be useful in developing a genetically engineered vaccine.

## Von Magnus Phenomenon and Defective Interfering Particles

Defective interfering particles accumulate at high multiplicities of infection

In early studies with influenza virus, it was noted that serial passage of virus stocks at high multiplicities of infection led to a steady decline of infectious titer with each passage. At the same time, the titer of noninfectious particles increased. As is discussed below, the noninfectious genomes interfere with the replication of the infectious virus and so are called **defective interfering (DI) particles.** Later, these observations were extended to include virtually all DNA as well as RNA animal viruses. The phenomenon is now named after von Magnus, who described the initial observations with influenza virus.

A combination of two separate events lead to this phenomenon. First, deletion mutations occur at a significant frequency for all viruses. For DNA viruses, the mechanisms are not well understood, but deletions presumably occur as a result of mistakes in replication or by nonhomologous recombination. The basis for the occurrence of deletions in RNA viruses is better understood. All RNA replicases have a tendency to dissociate from the template RNA but remain bound to the end of the growing RNA chain. By reassociating with the same or a different template at a different location, the replicase "finishes" replication, but in the process creates a shorter or longer RNA molecule. A subset of these variants possess the proper signals for initiating RNA synthesis and continue replicating. Because the deletion variants in the population require less time to complete a replication cycle, they eventually predominate and constitute the DI particles.

Deletions result from mistakes in replication, recombination, or the dissociation–reassociation of replicases

Second, as their name implies, the DI particles interfere with the replication of nondefective particles. Interference occurs because the DI particles successfully compete with the nondefective genomes for a limited supply of replication enzymes. The virions released at the end of the infection are therefore enriched for the DI particles. With each successive infection, the DI particles can predominate over the normal particles as long as the multiplicity of infection is high enough so that every cell is infected with at least one normal infectious particle. If this condition is satisfied, then the normal particle can complement any defects in the DI particles and provide all of the viral proteins required for the infection. Eventually, however, as serial passage is continued, the multiplicity of infectious particles drops below one, and the majority of the cells are infected only with DI particles. When this happens the proportion of DI particles in the progeny virus decreases.

Defective interfering particles compete with infectious particles for replication enzymes

In good laboratory practice, virus stocks are passaged at high dilutions to avoid the problem of the emergence of high titers of DI particles. Nevertheless, the presence of DI particles is a major contributor to the low fraction of infectious virions found in all virus stocks.

## Recombination

Besides mutation, genetic recombination between related viruses is a major source of genomic variation. Bacterial cells as well as the nuclei of animal cells contain the enzymes necessary for homologous recombination of DNA. Thus, it is not surprising that recombinants arise from mixed infections involving two different strains of the same type of DNA virus. The larger bacteriophages such as $\lambda$ and T4 code for their own recombination enzymes, a fact that attests to the importance of recombination in the life cycles and possibly the evolution of these viruses. The fact that recombination has also been observed for cytoplasmic poxviruses suggests that they too code for their own recombination enzymes.

Homologous recombination is common in DNA viruses

As far as is known, cells do not possess the machinery to recombine RNA molecules. However, recombination among at least some RNA viruses has been observed by two different mechanisms. The first, which is unique to the viruses with segmented genomes (orthomyxoviruses and reoviruses), involves reassortment of segments during a mixed infection involving two different viral strains. Recombinant progeny viruses that differ from either parent can be accounted for by the formation of new combinations of the genomic segments that are free to mix with each other at some time during the infection. Reassortment of this type occurring during infections of the same cell by human and certain animal influenza viruses is believed to account for the occasional drastic change in the antigenicity of the human influenza A virus. These dramatic changes, called **antigenic shifts**, produce strains to which much of the human population lacks immunity and, thus, can have enormous epidemiologic and clinical consequences (see Chapter 33).

Recombination for viruses with segmented RNA genomes involves reassortment of segments

Segment reassortment in mixed infections probably accounts for antigenic shifts in influenza virus

The second mechanism of RNA virus recombination is exemplified by the genetic recombination between different forms of poliovirus. Because the poliovirus RNA genome is not segmented, reassortment cannot be invoked as the basis for the observed recombinants. In this case, it appears that recombination occurs during replication by a "copy choice" type of mechanism. During RNA synthesis, the replicase dissociates from one template and resumes copying a second template at the exact place where it left off on the first. The end result is a progeny RNA genome containing information from two different input RNA molecules. Strand switching during replication, therefore, generates a recombinant virus. Although this is not frequently observed, it is likely that most of the RNA animal viruses are capable of this type of recombination.

Poliovirus replicase switches templates to generate recombinants

A "copy choice" mechanism has also been invoked to explain a high rate of recombination observed with retroviruses. Early after infection, the reverse transcriptase within

The diploid nature of retroviruses permits template switching and recombination during DNA synthesis

Occasional incorporation of host mRNA into retroviral particles may produce oncogenic variants

the virion synthesizes a DNA copy of the RNA genome by a process called reverse transcription. In the course of reverse transcription, the enzyme is required to "jump" between two sites on the RNA genome (see Chapter 42). This propensity to switch templates apparently explains how the enzyme generates recombinant viruses. Because reverse transcription takes place in subviral particles, free mixing of RNA templates brought into the cell in different virus particles is not permitted. However, retroviruses are diploid, because each particle carries two copies of the genome. This arrangement appears to be a situation ready-made for template switching during DNA synthesis and most likely accounts for retroviral recombination.

Occasionally, retroviruses package a cellular mRNA into the virion instead of a second RNA genome. This arrangement can lead to copy choice recombination between the viral genome and a cellular mRNA. The end result is sometimes the incorporation of a cellular gene into the viral genome. This mechanism is believed to account for the production of highly oncogenic retroviruses containing modified cellular genes (see below).

## THE LATENT STATE

The latent state involves infection of a cell with little or no virus production

Latent virus may be silent, change cell phenotype, or be induced to enter the lytic cycle

Temperate viruses can infect a cell and enter a latent state that is characterized by little or no virus production. The viral DNA genome is replicated and segregated along with the cellular DNA when the cell divides. There exist two possible states for the latent viral genome. It can exist extrachromosomally like a bacterial plasmid, or it can become integrated into the chromosome like the bacterial F factor in the formation of a high-frequency recombination (HFR) strain (see Chapter 4). Because the latent genome is usually capable of reactivation and entry into the lytic cycle, it is called a **provirus** or, in the case of bacteriophages, a **prophage.** In many cases, viral latency goes undetected; however, limited expression of proviral genes can occasionally endow the cell with a new set of properties. For instance, lysogeny can lead to the production of virulence-determining toxins in some bacteria (lysogenic conversion) and latency by an animal virus may produce oncogenic transformation.

## LYSOGENY

*E. coli* phage λ may be lytic or latent

When λ is integrated, the only active gene encodes a repressor for the other phage genes

Inactivation of repressor causes induction and virus production

Infection of an *Escherichia coli* cell by bacteriophage λ can have two possible outcomes. A portion of the cells (as many as 90%) enters the lytic cycle and produces more phage. The remainder of the cells enter the latent state by forming stable lysogens. The proportion of the population that lyses depends on as yet undefined factors including the nutritional and physiologic state of the bacteria. In the lysogenic state, the phage DNA is physically inserted into the bacterial chromosome (see below) and thus replicates when the bacterial DNA replicates. Lambda can thus replicate either extrachromosomally as in the lytic cycle or as a part of the bacterial chromosome in lysogeny. The only phage gene that remains active in a lysogen is the gene that codes for a repressor protein that turns off expression of all of the prophage genes except its own. This means that the lysogenic state can persist as long as the bacterial strain survives. Environmental insults such as exposure to ultraviolet light or mutagens, cause inactivation of the repressor, resulting in induction of the lysogen. The prophage DNA is excised from the bacterial chromosome, and a lytic cycle ensues.

Latent genomes can exist extrachromosomally or can be integrated

Once established, perpetuation of the lysogenic state requires a mechanism to ensure that copies of the phage genes are faithfully passed on to both daughter cells during cell division. Integration of the λ genome into the *E. coli* chromosome guarantees its replication and successful segregation during cell division. In bacteriophage P1 lysogens, the viral genome exists extrachromosomally as an autonomous single-copy plasmid. Its replication is tightly coupled to chromosomal replication and the two replicated copies are precisely partitioned along with the cellular chromosomes to daughter cells during cell division.

Phage λ integrates by site-specific recombination

Because of its mechanistic importance and relevance to lysogenic conversion and phage transduction (see Chapter 4), λ integration and the reverse reaction called excision are described in some detail. Bacteriophage λ integrates by a site-specific, reciprocal recombination event as outlined in Figure 7–1. There exist unique sequences on both the phage and

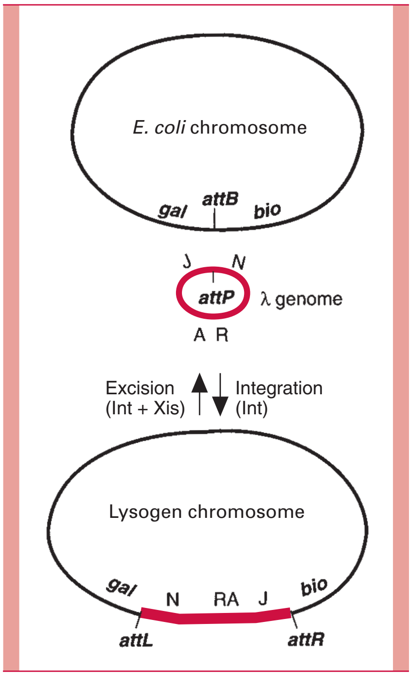


FIGURE 7–1
λ integration and excision. A, J, N, and R show the locations of some λ genes on the λ genome; *gal* and *bio* represent the *Escherichia coli* galactose and biotin operons, respectively.

bacterial chromosomes called attachment sites where the crossover occurs. The phage attachment site is called *attP* and the bacterial site, which is found on the *E. coli* chromosome between the galactose and biotin operons, is called *attB*. The recombination reaction is catalyzed by the phage-encoded integrase protein (Int) in conjunction with two host proteins and occurs by a highly concerted reaction that requires no new DNA synthesis.

Excision of the phage genome after induction of a lysogen is just the reverse of integration, except that excision requires, in addition to the Int protein, a second phage protein called Xis. In this case the combined activities of these two proteins catalyze site-specific recombination between the two attachment sites that flank the prophage DNA, *attL* and *attR* (see Fig 7–1). Early after infection, when integration is to occur in those cells destined to become lysogens, synthesis of the Xis protein is blocked. Otherwise, the integrated prophage DNA would excise soon after integration and stable lysogeny would be impossible. However, after induction of a lysogen, both the integrase and the Xis proteins are synthesized and catalyze the excision event that releases the prophage DNA from the chromosome.

Excision after λ induction involves recombination at junctions between host DNA and prophage

At a very low frequency, excision involves sites other than the *attL* and *attR* borders of the prophage and results in the linking of bacterial genes to the phage genome. Thus, if a site to the left of the bacterial *gal* genes recombines with a site within the λ genome (to the left of the J gene, otherwise the excised genome is too large to be packaged), then the resulting phage can transduce the genes for galactose metabolism to another cell (see Chapter 4). Similarly, transducing particles can be formed that carry the genes involved in biotin biosynthesis. Because only those cellular genes adjacent to the attachment site can be acquired by an aberrant excision event, this process is called **specialized transduction** to distinguish it from generalized transduction, in which virtually any bacterial gene can be transferred by a headful packaging mechanism (see Chapters 4 and 6).

Specialized transduction occurs because excision occasionally includes genes adjacent to the phage genome

Occasionally, one or more phage genes, in addition to the gene coding for the repressor protein, are expressed in the lysogenic state. If the expressed protein confers a new phenotypic property on the cell, then it is said that lysogenic conversion has occurred. Diphtheria, scarlet fever, and botulism are all caused by toxins produced by bacteria that have been "converted" by a temperate bacteriophage. In each case, the gene that codes for the toxin protein resides in the phage DNA and is expressed along with the repressor gene in the lysogenic state. It remains a mystery as to how these toxin genes were acquired by the phage; it is speculated that they may have been picked up by a mechanism similar to specialized transduction.

Lysogenic conversion results from expression of a prophage gene that alters cell phenotype

Several bacterial exotoxins are encoded in temperate phages

## MALIGNANT TRANSFORMATION

Malignant cells fail to respond to signals controlling the growth and location of normal cells

A tumor is an abnormal growth of cells. Tumors are classified as benign or malignant, depending on whether they remain localized or have a tendency to invade or spread by metastasis. Therefore, malignant cells have at least two defects. They fail to respond to controlling signals that normally limit the growth of nonmalignant cells, and they fail to recognize their neighbors and remain in their proper location. When grown in tissue culture in the laboratory, these tumor cells exhibit a series of properties that correlate with the uncontrolled growth potential associated with the tumor in the organism.

1. They have altered cell morphology.
2. They fail to grow in the organized patterns found for normal cells.
3. They grow to much higher cell densities than do normal cells under conditions of unlimited nutrients; therefore, they appear unable to enter the resting $G_0$ state.
4. They have lower nutritional and serum requirements than normal cells.
5. They have the capacity to divide in suspension, whereas normal cells require an anchoring substrate and grow only on surfaces (eg, glass or plastic).
6. They are usually able to grow indefinitely in cell culture.

Malignant transformation of cells in culture can be accomplished by most DNA viruses and some retroviruses

Many DNA animal viruses and some representatives of the retroviruses can convert normal cultured cells into cells that possess the properties listed above. This process is called **malignant transformation.** In addition to the listed properties, viral transformation usually, but not always, endows the cells with the capacity to form a tumor when introduced into the appropriate animal. Although the original use of the term **transformation** referred to the changes occurring in cells grown in the laboratory, current usage often includes the initial events in the animal that lead to the development of a tumor. In recent years, it has become increasingly clear that some but not all of these viruses also cause cancers in the host species from which they were isolated.

### Transformation by DNA Animal Viruses

Some oncogenic viruses cause tumors in their natural hosts

The oncogenic potential of animal DNA viruses is summarized in Table 7–1. All known DNA animal viruses, except parvoviruses, are capable of causing aberrant cell proliferation under some conditions. For some viruses, transformation or tumor formation has been observed only in species other than the natural host. Apparently infections of cells

TABLE 7–1

**Oncogenicity of DNA Viruses**

| Virus or Virus Group | Tumors in Natural Host[a] | Tumors in Other Species[b] | Transform Cells in Tissue Culture |
|---|---|---|---|
| Parvoviruses (rat, mouse, human) | No | No | No |
| Animal polyomaviruses (polyoma, simian virus 40) | No | Yes | Yes |
| Human polyomaviruses (JC, BK) | No | Yes | Yes |
| Papillomaviruses (human, rabbit) | Yes, often benign | ? | Yes |
| Human hepatitis B virus | Yes | ? | No |
| Human adenoviruses | No | Yes | Yes |
| Human herpesviruses | Yes | Yes | Yes |
| Poxviruses (human, rabbit) | Occasionally, usually benign | Yes | No |

[a] "Yes" means that at least one member of the group is oncogenic.
[b] Test usually done in newborns of immunosuppressed hosts.

from the natural host are so cytocidal that no survivors remain to be transformed. In addition, some viruses have been implicated in human or animal tumors without any indication that they can transform cells in culture.

In nearly all cases that have been characterized, viral transformation is the result of the continual expression of one or more viral genes that are directly responsible for the loss of growth control. Two targets have been identified that appear to be critical for the transforming potential of these viruses. Adenoviruses, papilloma viruses, and simian virus 40 all code for either one or two proteins that interact with the tumor suppressor proteins known as p53 and Rb (for retinoblastoma protein) to block their normal function which is to exert a tight control over cell cycle progression. The end result is endless cell cycling and uncontrolled growth.

In many respects, transformation is analogous to lysogenic conversion and requires that the viral genes be incorporated into the cell as inheritable elements. Incorporation usually involves integration into the chromosome (eg, papovaviruses, adenoviruses, and retroviruses), although the DNAs of some papillomaviruses and some herpesviruses are found in transformed cells as extrachromosomal plasmids. Unlike some of the temperate bacteriophages that code for the enzymes necessary for integration, papovaviruses and adenoviruses integrate by nonhomologous recombination using enzymes present in the host cell. The recombination event is therefore nonspecific, both with respect to the viral DNA and with respect to the chromosomal locus at which insertion occurs. It follows that for transformation to be successful, the insertional recombination must not disrupt a viral gene required for transformation. In summary, two events appear to be necessary for viral transformation: a persistent association of viral genes with the cell and the expression of certain viral "transforming" proteins.

Transformation by DNA viruses is analogous to lysogenic conversion

## Transformation by Retroviruses

Two features of the replicative cycle of retroviruses are related to the oncogenic potential of this class of viruses. First, most retroviruses do not kill the host cell, but instead set up a permanent infection with continual virus production. Second, a DNA copy of the RNA genome is integrated into the host cell DNA by a virally encoded integrase (IN); however, unlike bacteriophage λ integration, a linear form of the viral DNA, rather than a circular form, is the substrate for integration. Furthermore, unlike λ, there does not appear to be a specific site in the cell DNA where integration occurs.

Most retroviruses produce virions without causing host cell death

A DNA copy of the retroviral genome is integrated, but not at a specific site

Retroviruses are known to transform cells by three different mechanisms. First, many animal retroviruses have acquired transforming genes called **oncogenes.** More than 30 such oncogenes have now been found since the original oncogene was identified in Rous sarcoma virus (called v-*src*, where the v stands for viral). Because normal cells possess homologs of these genes called **protooncogenes** (eg, c-*src*, where c stands for cellular), it is generally thought that viral oncogenes originated from host DNA. It is possible they were picked up by "copy choice" recombination involving packaged cellular mRNAs as previously described. Because these transforming viruses carry cellular genes, they are sometimes referred to as **transducing** retroviruses. Most of the viral oncogenes have suffered one or more mutations that make them different from the cellular protooncogenes. These changes presumably alter the protein products so that they cause transformation. Although the mechanisms of oncogenesis are not completely understood, it appears that transformation results from inappropriate production of an abnormal protein that interferes with normal signaling processes within the cell. Uncontrolled cell proliferation is the result. Because tumor formation by retroviruses carrying an oncogene is efficient and rapid, these viruses are often referred to as **acute transforming viruses.** Although common in some animal species, this mechanism has not yet been recognized as a cause of any human cancers.

Retroviruses may carry transforming oncogenes

Oncogenes encode a protein that interferes with cell signaling

The second mechanism is called **insertional mutagenesis** and is not dependent on continued production of a viral gene product. Instead, the presence of the viral promoter or enhancer is sufficient to cause the inappropriate expression of a cellular gene residing in the immediate vicinity of the integrated provirus. This mechanism was first recognized in avian B-cell lymphomas caused by an avian leukosis virus, a disease characterized by a very long latent period. Tumor cells from different individuals were found to have a copy

Insertional mutagenesis causes inappropriate expression of a protooncogene adjacent to integrated viral genome of the provirus integrated at the same place in the cellular DNA. The site of the provirus insertion was found to be next to a cellular protooncogene called c-*myc*. The *myc* gene had previously been identified as a viral oncogene called v-*myc*. In this case, transformation occurs not because the c-*myc* gene is altered by mutation but because the viral promoter adjacent to the gene turns on its expression continuously and the gene product is overproduced. The disease has a long latent period; because, although the birds are viremic from early life, the probability of an integration occurring next to the c-*myc* gene is very low. Once such an integration event does occur, however, cell proliferation is rapid and a tumor develops. No human tumors are known for certain to result from insertional mutagenesis caused by a retrovirus; however, human cancers are known where a chromosome translocation has placed an active cellular promoter next to a cellular protooncogene (Burkitt's lymphoma and chronic myelogenous leukemia).

Human T-cell leukemia is caused by transactivating factor encoded in integrated HTLV-1

Transactivating factor turns on cellular genes, causing cell proliferation

The third mechanism was revealed by the discovery of the first human retrovirus. The virus, human T-cell lymphotropic virus type 1 (HTLV-1), is the causative agent of adult T-cell leukemia. HTLV-1 sequences are found integrated in the DNA of the leukemic cells and all the tumor cells from a particular individual have the proviral DNA in the same location. This observation indicates that the tumor is a clone derived from a single cell; however, the sites of integration in tumors from different individuals are different. Thus, HTLV-1 does not cause malignancy by promoter insertion near a particular cellular gene. Instead, the virus has a gene called *tax* that codes for a protein that acts in trans (ie, on other genes in the same cell) to not only promote maximal transcription of the proviral DNA, but also to transcriptionally activate an array of cellular genes. The resulting cellular proteins cooperate to cause uncontrolled cell proliferation. The *tax* gene is therefore different from the oncogenes of the acute transforming retroviruses in that it is a viral gene rather than a gene derived from a cellular protooncogene. HTLV-1 is commonly described as a **transactivating** retrovirus.

## ADDITIONAL READING

Natanson N. *Viral Pathogenesis and Immunity*. Philadelphia: Lippincott Williams & Wilkins; 2002. This readable, concise book covers viral pathogenesis, virus–host interactions, and host responses to infection. Specific topics include virulence, persistence, and oncogenesis.

# PART III

# HOST-PARASITE INTERACTIONS

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CHAPTER 8

# Immune Response to Infection

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Many innate defenses protect us from potential pathogens, including structural barriers and cells and molecules of the innate immune system such as phagocytes and acute phase proteins (also called inflammation), which are considered in Chapter 10. The **adaptive immune response** of vertebrates differs from these in that it is a specific, inducible, and anticipatory defense mechanism that allows the discrimination between self and nonself. The concept of immunity on which the science of immunology is built begins with ancient observations, such as Thucydides' description of the plague of Athens in 430 BC in which individuals who were infected and survived were not susceptible to infection by the same pathogen. A specific contemporary definition of the immune response is that it is a complex and precisely regulated inducible defense mechanism that allows the specific discrimination between self and nonself. The immune system requires for its function the presence of antigen-specific lymphocytes of two major types, thymus-derived lymphocytes (T cells) and bone marrow–derived lymphocytes (B cells), and it builds on the more primitive defense mechanisms of the **innate immune system** such as phagocytosis, while using mediators of cell communication termed **cytokines** to facilitate regulation of the complex system. Another characteristic that defines the immune response of mammals is that it is anticipatory; a process of combinatorial gene rearrangement generates an array of T and B cells with the aggregate populations comprising hundreds of millions of individual lymphocytes, each expressing a different receptor specificity in advance of any challenge. This preexisting readiness allows the production of circulating antibodies to the foreign challenge as well as the generation of the T-cell receptors that initiates the specific immune process leading to the elaboration of specific effector T lymphocytes (eg, helpers or killers).

Immunity discriminates between self and nonself

Specific mechanisms are inducible

One of the major recent successes of immunology has been eradication by vaccination of historic scourges such as smallpox. In addition to defense against infection, the immune system is important in normal developmental processes, aging, maintenance of internal homeostasis, and surveillance against neoplasms. This chapter presents an overview of major features of the immune system that are relevant to medical microbiology and infectious diseases. It is also intended to allow readers who have not yet studied immunology to understand the details of host–parasite interactions and immune responses to specific infections that are given elsewhere in the text. A listing of current immunology texts is provided at the end of the chapter.

Immune system is important for developmental processes and defense against infectious disease

The adaptive immune response differs from the innate or constitutive mechanisms in two major respects. The first is that the response is inducible; that is, the challenge to a healthy individual by a bacterium, virus, or other foreign (nonself) matter initiates a process

TABLE 8–1

## Cells Involved in the Immune System

| Cell | Function | Specific Receptors for Antigen | Characteristic Cell Surface Marker | Special Characteristics |
|---|---|---|---|---|
| B cells | Production of antibody<br>Present antigen to T cells | Surface immunoglobulin ($IgM_m$, $IgD_m$) | Fc and complement C3d receptors; MHC class II | Differentiate into plasma cells (major antibody producers) |
| T cells<br>Helpers ($T_H$) | Stimulate B cells by providing specific and nonspecific (cytokine) signals for activation and differentiation<br>Activate macrophages by cytokines | $\alpha/\beta$ Tcr | CD3+, CD4+, CD8− | Activation is restricted by MHC class II<br>Can be classified into two types: $T_H1$ activates macrophages, makes interferon $\gamma$, $T_H2$ activates B cells, makes IL-4 |
| Cytotoxic ($T_C$) | Lyse antigen-expressing cells such as virally infected cells or allografts | $\alpha/\beta$ Tcr | CD3+, CD4−, CD8+ | Restricted by MHC class I |
| Suppressors ($T_S$) | Downregulate cellular or humoral immunity | $\alpha/\beta$ Tcr, other variant Tcr | Can be CD3+ or CD3−; usually CD4−, CD8+ | |
| Regulatory T cell ($T_{REG}$) | Suppresses T cell–mediated inflammation | $\alpha/\beta$ Tcr | CD4+, CD25+ | Diminishes autoimmunity |
| Natural killer (NK) cells | Spontaneous lysis of tumor cells, antibody-dependent cellular cytotoxicity | Inhibitory (KIR); activating (eg, NKG2D) | Fc receptor for IgG | KIR recognize MHC class I |
| NK T cells | Amplify both cell-mediated and humoral immunity | $\alpha/\beta$ Tcr | CD4+ | Express a restricted subset of $V\alpha$ |
| Macrophages (monocytes) | Phagocytosis, secretion of cytokines to activate T cells (eg, IL-1) or other accessory cells such as neutrophils | None but can be “armed” by antibodies binding to Fc receptors | Macrophage surface antigens | Express surface receptors for the activated third component of complement (C3), kill ingested bacteria by oxidative bursts |
| Polymorphonuclear leukocytes (neutrophils, eosinophils) | Phagocytosis killing | None but can be “armed” by antibodies | | Protective in parasitic infections, but adverse side effects such as granuloma formation can occur |

*Abbreviations:* Tcr, T-cell receptor; MHC, major histocompatibility complex.

leading to the production of circulating proteins called **antibodies** that recognize and bind the invading pathogen in a specific manner. A second challenge by the same pathogen results in an accelerated immune response (secondary or anamnestic) that can confer greater protection on the host in a manner specific for that pathogen (eg, vaccination against measles protects against measles but not against polio).

Antibodies are inducible proteins that recognize and bind to invaders

The second major definitive characteristic of the human immune response is that it is anticipatory; that is, because of the combinatorial generation of the recognition repertoire, it has the potential to respond to pathogens not yet encountered in evolutionary history. This striking feature of the immune response results from the large number of genes specifying individual antibody combining sites for antigen and from a genetic recombination mechanism that allows us to form millions of potential antibody combining sites. Each antigen-specific lymphocyte (T or B) expresses a single receptor, and the cells are thus clonally restricted. In 1959, Sir MacFarlane Burnet predicted **clonal restriction** and **selection** by antigen, thus providing the intellectual foundation of modern immunology. The system is also endowed with the property of memory, so that reexposure to the inciting agent in the future usually brings about an enhanced response. Another crucial property of the combinational system is that it can be come **tolerant** or nonreactive to self based on contact during early development. Immune defenses against infectious organisms involve both the innate and adaptive systems, with emphasis on different aspects for individual pathogens.

Enormous capacity for diversity derives from clonal selection

Immune system has memory

## THE IMMUNORESPONSIVE CELLS

The function of the immune system requires antigen-specific lymphocytes of two major types (Table 8–1) and cytokines. **T cells** are thymus-derived lymphocytes and **B cells** are bone marrow–derived lymphocytes. **Cytokines** are secreted polypeptides that modulate the functions of cells (Table 8–2). Those produced by mononuclear cells (ie, lymphocytes and mononuclear phagocytic cells) are called **interleukins.** These regulate the growth and differentiation of lymphocytes and hematopoietic stem cells and the interactions among T cells, B cells, and monocytes in the elaboration of an immune response (see later discussion).

T cells, B cells, and mononuclear cells secrete peptides

T cells are responsible for (1) the initiation and modulation of immune responses (including B-cell responses); (2) cell-mediated immune processes that involve direct damage to antigen-bearing tissue or blood cells (eg, virally infected host cells); and (3) stimulation and enhancement of the nonspecific immune functions of the host (eg, the inflammatory reaction and antimicrobial activity of phagocytes). T cells are classified by the presence of the surface molecules called **CD4** and **CD8,** which in turn are related to functional activities classified as helper, suppressor, or cytotoxic.

T cells initiate and modulate immune responses and act directly

TABLE 8–2

**Biological Properties of Some Characterized Cytokines**

| Property | IFN-$\alpha$ | IFN-$\beta$ | IFN-$\gamma$ | IL-1$\alpha$ | IL-1$\beta$ | IL-2 | IL-3 | IL-4 | IL-5 | IL-10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Mitogenesis | | | + | + | + | + | + | + | + | + |
| Effect on macrophages | + | + | + | | | + | | + | | + |
| B-cell activation | | | + | + | + | | | + | + | |
| B-cell proliferation | + | + | + | + | + | + | + | + | ? | |
| B-cell differentiation | + | | + | + | + | + | + | + | ? | + |
| Ig isotype selection | | | | | | | | IgE: IgG1 | IgA | + |
| T-cell activation | | | | + | + | + | | + | | |
| T-cell proliferation | + | | | + | + | + | | + | | + |
| T-cell differentiation | | | | | | + | | + | | |
| Pyrogenic | + | + | + | + | + | + | | | | |

B cells are responsible for humoral immunity

T and B cells are widely distributed

B cells are responsible for humoral immunity through antibody production. Individual B cells have antibody of a single specificity on their surface that can bind directly to foreign antigens. B cells can also differentiate into plasma cells, which produce a soluble antibody that can circulate in blood and body fluids independent of cells. T and B cells are found throughout the body, particularly in the bone marrow; specialized areas of the lymph nodes and spleen; lymphoid structures adjacent to the alimentary and respiratory tracts (eg, Peyer's patches and adenoids); and subepithelial tissues of the internal organs. They are continually replaced, and there is considerable circulation of B and T cells between the different areas of the body through the lymphatic and blood vascular circulations.

## ANTIGENS AND EPITOPES

Antigens stimulate and react with antibody

Epitopes fit to the combining site of T-cell receptors and antibodies

An **antigen** is a substance (usually foreign) that reacts with antibody and may stimulate an immune response when presented in an effective fashion. A large structure such as a protein, virus, or bacterium contains many subregions that are the actual antigenic determinants, or **epitopes.** These epitopes can consist of peptides, carbohydrates, or particular lipids of the correct size and three-dimensional configuration to fill the combining site of an antibody molecule or a T-cell receptor (Fig 8–1). Approximately six amino acids or monosaccharide units provide a correctly sized epitope. Much of our knowledge of the combining sites of antibodies and their specificities was determined by immunizing animals experimentally with small organic molecules called **haptens.** Some of the best examples of these are substituted phenols, such as 2,4-dinitrophenol, which themselves do not induce the production of antibodies but must be coupled to a **carrier molecule** to be immunogenic. The term **immunogen** is a synonym for antigen, but it is sometimes restricted to those antigens able to elicit an immune response as distinguished from the ability to react only with antibodies and with T-cell receptors.

B cells multiply and produce antibody

A foreign antigen entering a human host may by chance encounter a B cell whose surface antibody is able to bind it. This interaction stimulates the B cell to multiply, differentiate, and produce more surface and soluble antibody of the same specificity. Eventually, the process leads to production of enough antibody to bind more of the antigen. This mechanism is most likely to operate with antigens such as polysaccharides that have repeating subunits, thus improving the chance that exposed epitopes are recognized.

Protein antigens must be processed first

Large, complex antigens such as proteins and viruses must be processed before their epitopes can be effectively recognized by the immune system. This processing takes place in macrophages or specialized epithelial cells found in the skin and lymphoid organs,

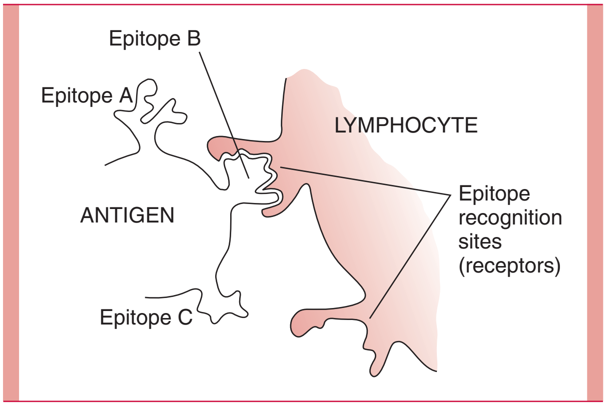


FIGURE 8–1
Schematic of epitope recognition by an immunoresponsive lymphocyte. Epitope B on the antigen binds to a complementary recognition site on the surface of the immunoresponsive cell. Antigens may have multiple different epitopes, but an immunoresponsive lymphocyte has receptors of only one specificity. In most cases, epitopes are recognized on the surface of macrophages that have processed the antigen. The receptor for antigens on B cells is the combining site of the surface immunoglobulin.

where they are adjacent to other immunoresponsive cells. The ingested antigen is degraded to peptides of 10 to 20 amino acids that are presented by major histocompatibility (MHC) products on the host cell surface to be recognized by T cells.

## BASIS OF IMMUNOLOGIC SPECIFICITY

The intellectual framework for understanding the mechanisms of immunologic specificity was laid down by the theory of **clonal selection.** It is now generally accepted that human lymphocyte populations, both B and T cells, show a great heterogeneity inasmuch as different cells possess surface receptors, which differ from each other with respect to combining site. This is shown in Figure 8–2 for B cells. In the actual process, great heterogeneity in the immune response even to particular antigens is observed. Particular domains termed **hypervariable regions** provide the actual amino acid residues that confer individual specificity. In the role of B cells in antibody production, there would be a differentiation from the lymphocytes to the plasma cells, and shifts of types of antibody would occur, depending on secondary stimulation and regulatory cytokines.

Clonal selection provides diversity in amino acid residues

With the elimination of antigen, the majority of the clone of immunoreactive lymphocytes is lost over time by normal cell replacement. However, the speed with which antigen is lost is very variable and depends on such factors as excretion and enzymatic breakdown. Some polysaccharide antigens and bacterial cell wall peptidoglycans are so resistant to host enzymatic breakdown that they can persist for years, whereas many protein antigens are rapidly metabolized. Fortunately, the immune system has a recall

Some antigens persist for years

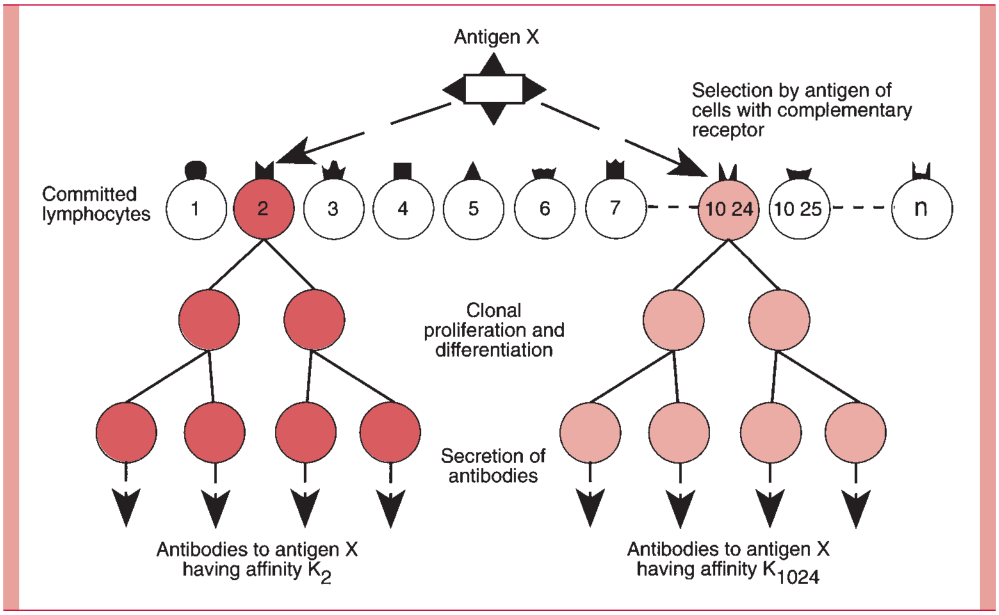


FIGURE 8–2
Diagram of the cellular events involved in the clonal selection of specifically reactive B lymphocytes by antigen. Clonal selection of T lymphocytes could be depicted by a comparable scheme, but T cells do not secrete antibodies and the antigen would be presented in association with molecules of the major histocompatibility complex. Each B cell is numbered to show that it represents an individual clone. The schematic representation of the surface immunoglobulin receptors indicates that these have distinct combining sites. The combining sites are formed by interaction of $V_H$ and $V_L$ domains, and the cell-to-cell distinction in receptor specificity results from essentially a random genetic process. If a particular antigen, designated X here, enters the system, it can bind specifically, albeit with different affinities, to two of the cells shown here. If there are proper antigen presentation and interplay of cytokines involved in activation in differentiation, the recognition of antigen by the surface immunoglobulin receptor results in clonal proliferation and differentiation of those cells recognizing the antigen. In this case, antibodies representing two types of combining sites are generated.

Memory cells survive to provide recall ability

ability in the case of protein antigens, because certain cells in the clone, termed **memory cells,** survive long periods and probably slowly replicate to maintain a core population with the capacity to expand very rapidly if the antigen (or the same epitope on another antigen) is encountered again.

Secondary response is rapid and greater than primary response

Vaccines stimulate secondary responses

Memory cells may be either T or B cells and are probably variants within the original clone having recognition sites with higher specific affinity for the relevant antigenic determinant and, thus, greater immunologic efficiency. As a consequence, the response to a second encounter with an antigen is more rapid than the first and quantitatively greater in its effect. It is referred to as a secondary response, in contrast to the initial primary response. Memory cells and the secondary response phenomenon account for the prolonged or lifelong immunity that follows many infections (eg, measles), and the secondary response is exploited in scheduling doses of various vaccines to obtain the maximum and most long-lived immunity.

## THE T-CELL RESPONSE

The major roles of T cells in the immune response are:

1. Recognition of peptide epitopes presented by MHC molecules on cell surfaces. This is followed by activation and clonal expansion of T cells in the case of epitopes associated with class II MHC molecules.
2. Production of lymphokines that act as intercellular signals and mediate the activation and modulation of various aspects of the immune response and of nonspecific host defenses.
3. Direct killing of foreign cells, of host cells bearing foreign surface antigens along with class I MHC molecules (eg, some virally infected cells), and of some immunologically recognized tumor cells.

### Antigen-Specific Receptors of T Cells

$\alpha/\beta$ and $\gamma/\delta$ T-cell receptors are associated with CD3 complex

There are two major types of T-cell receptors in humans. More than 90% of T cells in adult spleen, lymph nodes, and peripheral blood express the $\alpha/\beta$ receptor, which is depicted in Figure 8–3. A small subset (usually 5%) of T cells express the $\gamma/\delta$ receptor. The $\gamma/\delta$ receptor is more prevalent on fetal T cells, has a limited capacity for diversity, and shows an association with responses to mycobacterial infections. Both the $\alpha/\beta$ and $\gamma/\delta$ T-cell receptors occur in association with the CD3 complex, a set of at least five distinct proteins that is necessary for signal transduction and allows activation of the T cells following recognition of antigen.

MHC presents processed peptides to the T-cell receptor

MHC class II are only on lymphocytes and macrophages

A particular set of cell surface proteins specified by the genes of the MHC plays a major role in the recognition of antigens by T cells. These were first discovered through transplantation experiments, where it was found that they were major markers recognized in graft rejection. Subsequently, a strong association between susceptibility to disease and particular MHC markers was found. The MHC contains sets of genes that are designated as class I and class II determinants. These loci are highly polymorphic, and within populations, association with particular MHC markers correlates with the capacity to respond to particular antigens. Recent studies have shown that peptide determinants produced by proteolytic degradation of proteins by antigen-presenting accessory cells bind to MHC products, which then present the peptide antigen to the $\alpha/\beta$ T-cell receptor. Human class I molecules (HLA-A, HLA-B, and HLA-C) are expressed on virtually all cells of the body, whereas class II molecules (HLA-DR) are restricted to lymphocytes and macrophages, including important antigen-presenting cells such as dendritic cells.

CD4 and CD8 surface markers vary on T cells

Cytotoxic T cells recognize antigen on MHC class I molecules and express the CD8 marker. By contrast, cells bearing the $\gamma/\delta$ antigen-specific T-cell receptor (Tcr) lack both CD4 and CD8. Figure 8–3 shows a membrane form of antibody expressed on B cells as a comparison with the $\alpha/\beta$ antigen-specific receptor of T cells. $\alpha/\beta$ T-cell receptors have not been found to any degree in serum and exist predominantly as cell surface recognition molecules. The affinity of T-cell receptors for antigen is low, and the role of MHC presentation of antigen is most probably to compensate for the low affinity.

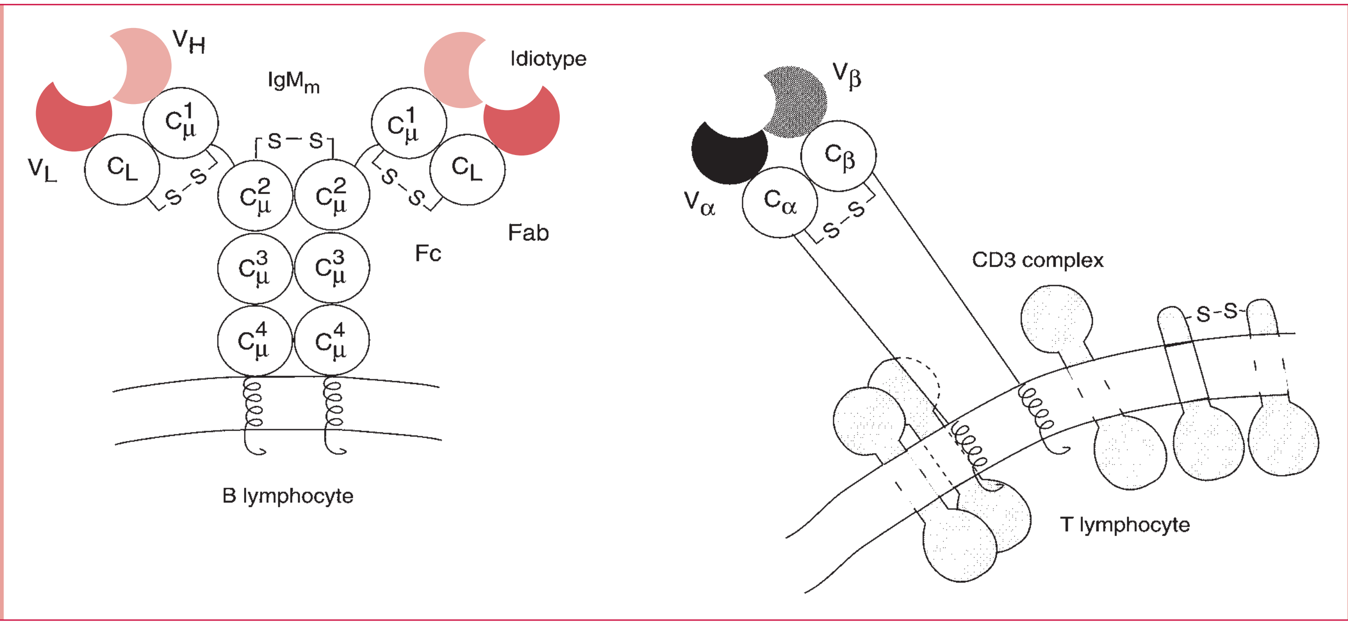


FIGURE 8–3
Comparison of the membrane IgM receptor of primary B cells with the $\alpha/\beta$ T-cell receptor of helper, cytolytic, and delayed-type hypersensitivity T cells. The $IgM_m$ molecule is a monomer consisting of two $\mu$ chains and two light chains, in contrast with the pentamer shown in Figure 8–6. The locations of the combining sites for antigen and the idiotypic marker are depicted. An additional difference between the serum form and the membrane form is the presence of a helical transmembrane region at the C-terminal end of the membrane receptor. In overall form, the $\alpha/\beta$ T-cell receptor is a disulfide-bonded heterodimer that resembles a single Fab fragment of immunoglobulin. In addition, it has an elongated stretch comparable to a hinge that terminates in a membrane-spanning helical region.

Specific T-cell help is initiated by the binding of $\alpha/\beta$ Tcr to antigen presented by MHC class II molecules. Most of the details of antigen presentation have been established using protein antigens, with an emphasis on virally infected cells so that the general principles apply to specific cytolytic cells as well. These proteins are digested into peptides by phagocytic cells (sometimes referred to as accessory cells), with certain peptides bound in a peptide-binding cleft within the MHC molecules intracellularly. These peptide–MHC complexes are then expressed on the cell surface, where the peptide epitope can be presented to the low-affinity antigen combining site of the $\alpha/\beta$ Tcr on T cells of compatible MHC type.

Peptides digested in phagocytes are presented to T cells

The initial specificity of cytotoxic T cells is, likewise, impacted by the $\alpha/\beta$ Tcr, but the MHC restriction involves class I molecules. In humans, the vast majority of circulating T cells bear the $\alpha/\beta$ Tcr. The CD3 surface marker comprises at least five distinct proteins involved in forming a membrane activation complex in association with the Tcr. The $\gamma/\delta$ Tcr is the first to appear in fetal development, but it constitutes less than 5% of T cells in the adult. Like the $\alpha/\beta$ Tcr, it occurs in association with CD3. $\gamma/\delta$ Tcr-bearing T cells are cytotoxic but do not show MHC restriction.

Surface CD3 associates with Tcr

Other cellular phenomena can be nonspecific in the sense that neither antigen-specific antibodies nor T-cell receptors are involved. These include natural killer (NK) cells and armed or activated macrophages such as mast cells, basophils and eosinophils, and macrophages. Activated macrophages produce substances toxic to intracellular pathogens, including reactive nitrogen and oxygen intermediates that can kill the organisms. NK cells are cells related to lymphocytes that are present in the absence of antigenic stimulation that recognize and kill particular types of turnor cells. NK cells recognize MHC class I through inhibitory receptors and lyse cells such as tumors that lack the MHC markers. Two major types of NK cells have been described: NK cells and NKT cells. The first type are usually large and granular and kill certain tumors and also function in innate immunity to viruses

NK cells do not require antigen stimulation

Innate viral immunity is related to NK cells and intracellular pathogens. NKT cells express the $\alpha/\beta$ Tcr but with only one V$\alpha$ gene product, have T-cell markers, and are secreted by $T_H1$ (interferon-$\gamma$, or IFN-$\gamma$) and $T_H2$-type (interleukin-4, or IL-4) cytokines.

Cytotoxic T cells are antigen-specific killer cells

Antigen-specific sensitized killer cells are induced by specific sensitization with the target antigen. The cytotoxic T cells are activated by the presence of processed antigen and cytokines by a MHC-compatible antigen-presenting cell and show a subsequent MHC restriction in their capacity to destroy target cells. Cytotoxic T cells use the $\alpha/\beta$ T-cell receptor in recognition of peptide I MHC antigens. Cells with delayed-type hypersensitivity also are antigen-specific T cells that can be generated in the absence of circulating antibodies. An example of delayed-type hypersensitivity is skin sensitization with small organic molecules, such as the quinone produced by poison ivy.

## CD4+ Helper T Lymphocytes

$T_H$ cells are stimulated by MHC II presented antigen

IL-1 then IL-2 are secreted

B-cell differentiation is triggered

Helper T cells ($T_H$ cells) are stimulated by antigen in the context of MHC class II presentation and are further marked by the presence of the CD4 cell surface antigen. If T cells are of the proper MHC background to recognize the antigen specifically, T-cell activation occurs in the presence of IL-1. The antigen–MHC complex presented to a specific T cell by the macrophage is the specific signal that induces the T cell to become activated and divide. The secretion of IL-2, following stimulation by IL-1, promotes the division of T cells following contact with antigens. The activated $T_H$ cell presents both antigen and regulatory cytokines to the B cells, orchestrating the scheme of B cell differentiation from small lymphocytes to plasma cells producing antibodies of various types. The ability of particular B cells or T cells to respond to stimulation by individual cytokines is dependent on the presence of surface receptors for those cytokines.

Cytokines regulate physiologic processes

Role of $T_H1$ and $T_H2$ varies with antibody, cells, and infectious agents

Table 8–2 outlines the biological properties of some characterized cytokines. Cytokines can be involved in general physiologic or aphysiologic processes such as the induction of fever, mitogenesis or division of lymphocytes, and the stimulation of phagocytic cells. Other cytokines are involved in regulating activation of specific subsets of lymphocytes, and some have an extremely specific function in regulating the immunoglobulin isotypes expressed. The immune response is a complex but precisely regulated defense system in which specific recognition is imparted by antibodies, B-cell immunoglobulin receptors, and T-cell receptors, and activation and differentiation are dependent on a regulatory cascade of cell–cell communication molecules. The functional roles of cytokines produced by subsets of CD4+ helper cells; $T_H1$ and $T_H2$, are essential for the discrimination between antibody formation ($T_H2$) and inflammatory cell-mediated immunity ($T_H1$) and, consequently, for the severity of autoimmune disease ($T_H1$), the capacity to reject tumors ($T_H1$), the immune resistance to viruses and intracellular parasites ($T_H1$), the resistance to helminth worms ($T_H2$), the susceptibility to viruses and intracellular parasites ($T_H2$), and susceptibility to allergic disorders ($T_H2$).

HIV binds to CD4 molecule

The critical significance of CD4+ helper cells to the body is shown by the catastrophic effects of acquired immunodeficiency syndrome (AIDS), in which the human immunodeficiency virus (HIV) binds to the CD4 molecule, enters the cell, and interferes with its function or destroys it. As a result, the body becomes susceptible to a wide variety of bacterial, viral, protozoal, and fungal infections, both through loss of preexisting immunity and through failure to mount an effective immune response to newly acquired pathogens.

## CD8+ Cytotoxic T Lymphocytes

CD8+ lymphocytes react with MHC I

Eliminate virally infected cells

CD8+ cytotoxic T lymphocytes are a second class of effector T cells. They are lethal to cells expressing the epitope against which they are directed when the epitope is in conjunction with class I MHC molecules. They too have specific epitope recognition sites, but they are characterized by the CD8 cell surface marker; thus, they are referred to as CD8+ cytotoxic T cells. These cells recognize the association of antigenic epitopes with class I MHC molecules on a wide variety of cells of the body. However, this recognition does not itself lead to the necessary clonal expansion of CD8 cells, which also requires the lymphokine IL-2 to be produced by activated CD4+ lymphocytes. In the case of virally infected cells, cytotoxic CD8+ cells prevent viral production and release by eliminating the host cell before viral synthesis or assembly is complete.

### CD8+ Suppressor T Cells

Suppressor T lymphocytes also carrying the CD8 marker and epitope recognition sites are involved in modulating and terminating the immunologic activities of both T and B cells, thus avoiding excessive or needlessly prolonged responses that could interfere with other immunologic activities. It is known that the suppression they produce may be antigen specific or it may be polyclonal (ie, affecting general immunologic responses irrespective of the inciting antigen). The mechanisms of suppression and control are less well defined than are the activities of CD4+ helper cells. In AIDS, the proportion of CD8+ suppressor cells relative to CD4+ helper T cells is substantially increased, because CD8+ lymphocytes are not attacked by HIV. This imbalance, in addition to the depletion of CD4+ helper cells, may contribute to the immunosuppression that is characteristic of the disease.

Suppressor T cells modulate T- and B-cell activities

Spared by HIV

### Regulatory T Cells

Regulatory T cells are CD4+, Tcr $\alpha,\beta$+ T cells that also express the CD25 marker. They suppress $T_H1$-type mediated inflammatory responses, particularly destructive autoimmunity.

Autoimmunity is suppressed

### Response to Superantigens

A group of antigens have been termed **superantigens** because they stimulate a much larger number of T cells than would be predicted based on the generation of combining site diversity through clonal selection. Superantigens activate 3 to 30% of T cells in unstimulated animals. The action of superantigens is based on their ability to bind directly to MHC proteins and to particular V$\beta$ regions of the T-cell receptor (see Fig 8–3) without involving the antigen combining site. Individual superantigens recognize exposed portions defined by framework residues that are common to the structure of one or more V$\beta$ regions. Any T cells bearing those V$\beta$ sites may be directly stimulated. A variety of microbial products have been identified as superantigens. An example in which the pyrogenic exotoxins of *Staphylococcus aureus* and group A streptococci act as superantigens is **toxic shock syndrome** (see Chapters 16 and 17).

Superantigens bind directly to MHC proteins and Tcr V$\beta$ region

Higher proportion of T cells are stimulated

## CELL-MEDIATED IMMUNITY

Cell-mediated immunity is most dramatically expressed as a response to obligate or facultative intracellular pathogens. These include certain slow-growing bacteria, such as the mycobacteria, against which antibody responses are ineffective. In experimental infections, cell-mediated immunity can be passively transferred from one animal to another by T lymphocytes but not by serum. (In contrast, short-term, antibody-mediated [B-cell] immunity can be passively transferred with serum.) The mechanisms of cell-mediated immunity are complex and involve a number of cytokines with amplifying feedback mechanisms for their production. The initial processing of antigen is accompanied by sufficient IL-1 production by the macrophages to stimulate activation of the antigen-recognizing CD4+ (helper) cell. Lymphokine feedback from the CD4+ T cells to macrophages further increases IL-1 production. IL-2 produced by the CD4+ T cells facilitates their clonal expansion and activates CD8+ (cytotoxic) T lymphocytes. Other lymphokines from CD4+ T cells chemotactically attract macrophages to the site of infection, hold them there, and activate them to greatly enhance microbicidal activity. The sum of the individual and collaborative activities of T cells, macrophages, and their products is a progressive mobilization of a range of nonspecific host defenses to the site of infection and greatly enhanced macrophage activity. In the case of viruses, IFN-$\gamma$ inhibits replication, and CD8+ cytotoxic lymphocytes destroy their cellular habitat, leaving already assembled virions accessible to circulating antibody. The interplay among cells of the innate immune system, including monocytes, macrophages, and dendritic cells; the essential elements of specific immune system, T cells (particularly $T_H1$ and $T_H2$ cells), B cells, and antibodies; and the regulatory roles of proinflammatory (eg, IL-2, IFN-$\gamma$)

Of primary importance with intracellular pathogens

Helper and cytotoxic T lymphocytes interact

Macrophages are mobilized and enhanced

and anti-inflammatory (eg, IL-4, IL-6) cytokines in adaptive resistance to particular types of pathogenic organisms will be considered below.

Prolonged cell-mediated immune response may cause injury

With certain infections in which reaction to protein antigens is particularly strong (eg, in the response to *Mycobacterium tuberculosis*), the cell-mediated responses are of such magnitude that they become major deleterious factors in the disease process itself. This is called delayed-type hypersensitivity, because reexposure of the host to the antigen that elicited the immune response produces a maximum hypersensitive reaction only after a day or two, when mobilization of immune lymphocytes and of phagocytic macrophages is at its peak.

## B CELLS AND ANTIBODY RESPONSES

B cells carry epitope recognition sites on their surface

Stimulated cells differentiate to form plasma cells

B lymphocytes are the cells responsible for antibody responses. They develop from precursor cells in the yolk sac and fetal liver before birth and thereafter in the bone marrow before migrating to other lymphoid tissues. Each mature cell of this series carries a specific epitope recognition site on its surface—the antigen-recognizing (variable) region of antibody that will be produced subsequently by its progeny. In the process of antibody formation, B lymphocytes, following stimulation by antigen, divide and differentiate into plasma cells, which are end cells adapted for secretion of large amounts of antibodies. In addition to their essential role in antibody production, B cells can present antigen to T cells.

T-cell independent responses require only polysaccharide antigen and B cells

T-cell dependent responses require helper T cells

T-independent responses develop more slowly from birth

There are two broad types of antigens. **T-independent antigens** are those that do not require help by T cells to stimulate B-cell antibody production, and **T-dependent antigens** are those that are dependent on collaboration between helper T cells and B cells to initiate the process of antibody production. T-independent antigens are generally limited to large polymeric molecules such as carbohydrates with repeating sugar epitopes. Antibodies are particularly effective and essential to the protective immune response to the polysaccharide capsule of *Streptococcus pneumoniae,* because these bacteria would not otherwise be bound and ingested by phagocytes. Killing of the bacteria is initiated by the specific binding of antibodies to the surface polysaccharides, and it is carried out by either the binding of complement to the antibody on the bacterial surface or by the binding of Fc receptors on phagocytic cells to the bound antibody, thus facilitating ingestion and intracellular killing. Immunologic reactivity to such polysaccharides usually develops much more slowly after birth than do the T-dependent responses, and memory cells do not result from the clonal B-cell expansion. This delay in responsiveness probably contributes to the increased susceptibility to some bacterial infections in early life. Most common antigens, particularly proteins, require T-cell help by CD4+ cells for antibody production to occur. Following stimulation by antigen processed and presented by macrophages, T cells can become helper cells collaborating with B cells, antigen-specific cytotoxic T cells capable of killing tumor cells, suppressor T cells downregulating the immune system, or T cells mediating delayed-type hypersensitivity. Table 8–1 lists major cells in the immune response and their antigen-specific and nonspecific functions.

Antigen processing causes delay in antibody response

Learning system increases affinity with time or secondary challenge

Following challenge with foreign antigen, there is a lag period of 4 to 6 days before antibody can be detected in serum. This period reflects the events involved in the recognition of the antigen, its processing, and the specific activation of the cells of the immune system. The first event is the clearance of antigen from the circulation by what is essentially a metabolic process in which the antigen is recognized in a nonspecific sense and ingested. The vast preponderance of antigen ends up in circulating phagocytes or in stationary macrophages such as the Kupffer cells in the liver. The macrophages process the antigen so that immunogenic moieties can be presented to T cells (Fig 8–4). IL-4, IL-5, and IL-6, in addition to specific presentation of antigen, cause the B cells to produce immunoglobulins and also are involved in class switches. The antibody-forming system is a learning system that responds to challenge by foreign molecules by producing large amounts of specific antibody. In addition, the affinity of its binding to the specifically recognized antigen often increases with time or secondary challenge.

### Antibodies

Antibodies belong to the **immunoglobulin** family of proteins, which occur in quantity in serum and on the surfaces of B cells. The basic structure of an immunoglobulin is illustrated

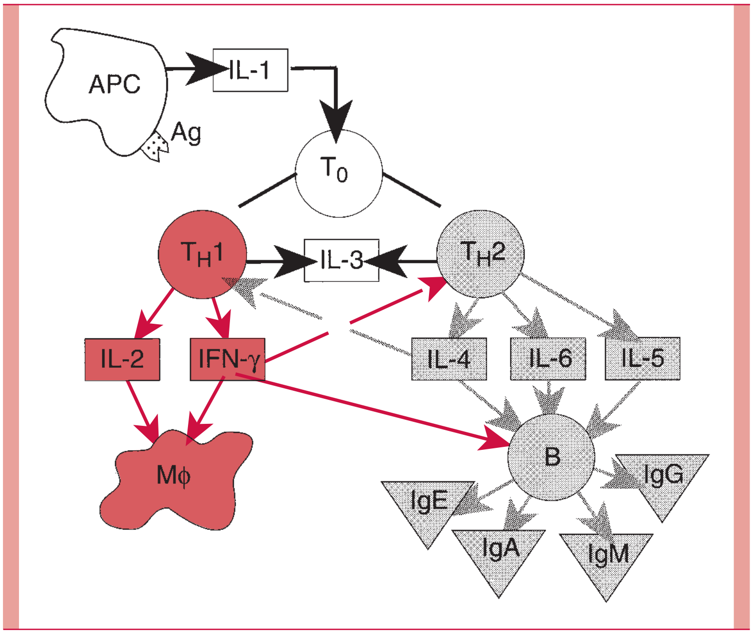


FIGURE 8–4
Simplified diagram illustrating events of helper T-cell activation leading to either cellular immunity of the delayed-type hypersensitive type ($T_H1$ cells) or antibody production ($T_H2$) involving stimulation of B cells by specific and nonspecific (cytokine) means. The cytokine interleukin-2 (IL-2) plays a major role in causing T-cell mitogenesis and also in the activation of macrophages. This is abbreviated as an antigen-presenting cell (APC), which presents both processed antigen (Ag) to antigen-specific T cells and a stimulatory cytokine (IL-1) that causes the stimulated T cell to differentiate into one of two broad types of T cells; termed $T_H1$ and $T_H2$ here. The $T_H1$ cells produce IL-2 and IL-3 and interferon-$\gamma$ and can stimulate macrophages, $T_H2$ cells, and B cells. The $T_H2$ cells produce IL-3, -4, -5, and -6 and carry out a major role as helpers in activating B cells.

in Figure 8–5, which depicts an IgG molecule. Immunoglobulins have a basic tetrameric structure consisting of two light polypeptide chains and two heavy chains usually associated as light/heavy pairs by disulfide bonds. The two light/heavy pairs are covalently associated by disulfide bonds to form the tetramer. There are two types of light chains, $\kappa$ chains and $\lambda$ chains, which are the products of distinct genetic loci. The class or isotype of the immunoglobulin is defined by the type of heavy chain expressed. In this IgG molecule, the heavy chains are termed $\gamma$ chains and have characteristic sequences and antigenic markers. IgG immunoglobulins can have either $\kappa$ or $\lambda$ chains associated with the $\gamma$, but only one type of light chain would be present in the intact molecule. That is, an individual IgG molecule would be either $\gamma2\kappa2$, or $\gamma2\lambda2$; mixed molecules do not occur. This diagram illustrates other basic structural features of the molecule. The basic building block of immunoglobulins is a domain of approximately 110 amino acids containing an internal disulfide bond stabilizing the structure. **Domains** are compact, tightly folded structures having a characteristic "immunoglobulin fold." The light chains contain two domains: a variable domain and a constant domain. The $\gamma$ heavy chain contains four domains: $V_H$, $C_\gamma1$, $C_\gamma2$, and $C_\gamma3$.

Immunoglobulins have tetrameric structure combining light chains and heavy chains

Isotypes are defined by type of heavy chain

Antibodies carry out two broad sets of functions: the recognition function is the property of the combining site for antigen, and the effector functions are mediated by the constant regions of the heavy chains. Antibodies combine with foreign antigens, but the actual destruction or removal of antigen requires the interaction of portions of the Fc fragment with other molecules such as complement components or with effector cells, which then engulf the recognized cell or particle.

Antibodies have recognition and effector functions

The combining site for antigen (antigen binding site) is formed by interaction of the **variable domains** of the heavy chain and the light chain. The IgG molecule has two such combining sites. Immunoglobulin in the serum of normal individuals occurs as a large pool of individual molecules, each of which has a unique sequence and a defined combining

Combining site is idiotype

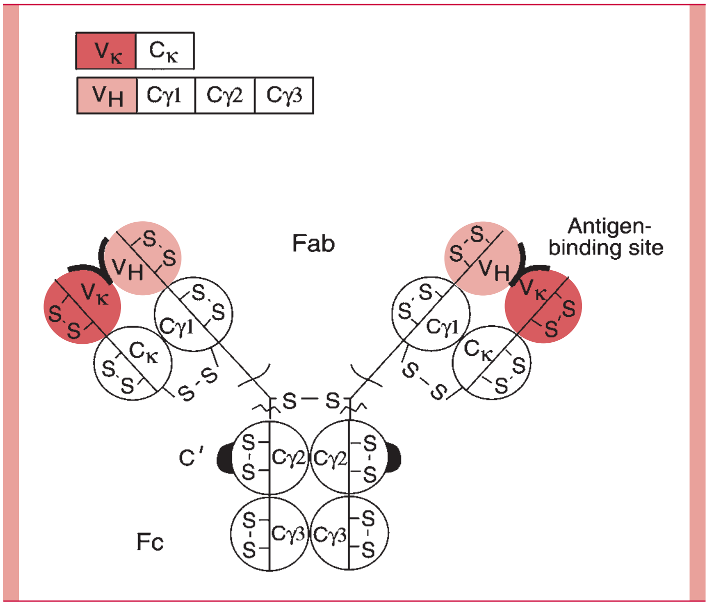


FIGURE 8–5
Schematic representation of an IgG immunoglobulin molecule. This model illustrates the domain structure of immunoglobulin light and heavy chains in a stick model form (top) and as compact, circular domains (bottom). Two combining sites for antigen are present, and these are formed by interaction between the $V_H$ and $V_K$ domains of the molecule. The binding site for complement (C′) is shown to be located in the C$\gamma$2 domain. The region of the heavy chain where no domain structure is shown is the "hinge" region. This region is the site of cleavage of the proteolytic enzymes papain and pepsin. The Fc fragment produced by proteolysis contains the binding site for complement and is crystallizable. The Fab fragment contains the variable regions and binds antigen.

site. The defined combining site of an immunoglobulin has been termed an **idiotype.** The idiotype is a combining site-related antigenic marker that defines individual immunoglobulins. Other types of antigenic markers of immunoglobulins define classes or isotypes. These occur on the constant regions of light chains, where they define the $\kappa$ and $\lambda$ isotypes. Heavy chains have $C_H$ markers identifying $\mu$, $\gamma$, $\delta$, $\alpha$ and $\varepsilon$ isotypes. These markers are found in all normal individuals. The third general type of immunoglobulin antigenic determinant is termed **allotypic.** These markers may be found on the light chains (eg, the KM determinant of human $\kappa$ chains) or heavy chains (eg, the GM markers of human IgG) and define genetic markers that behave as Mendelian alleles in the human population. Allotypic markers are usually associated with constant regions but have been reported for the variable domains of heavy chains as well.

Allotype markers are in light or heavy chains

Another structural feature of immunoglobulins that merits consideration is the fact that proteolytic digestion of the IgG molecule by the enzyme papain can cleave the structure into two defined regions. As illustrated in Figure 8–5, two **antigen-binding** or **Fab** fragments are generated, and a single **constant** or **Fc** fragment is produced. The cleavage occurs in the so-called hinge region, which is a relatively loose stretch of polypeptide connecting the Fab domains to the $C_\gamma 2$ domain. The tight domain structures themselves are relatively resistant to proteolysis. The positions of the intradomain disulfides are indicated (S-S bonds). The intrachain disulfide bonds connecting the $C_\kappa$ and $C_\gamma 1$ are also indicated, as is the location of the S-S bonds linking the two heavy chains. This basic structure, although using different heavy chains, occurs in all five of the major human immunoglobulin classes, but the number of subunits and the overall arrangement can vary. For example, Figure 8–6 gives a schematic representation of a serum IgM immunoglobulin. This molecule, which was originally called immune macroglobulin because of its large size (approximate mass of 900,000 daltons), consists of five subunits of the form of the typical IgG. The light chains can be either $\kappa$ or $\lambda$, but the type of heavy chain defining the IgM class is termed the $\mu$ chain. The molecule occurs as a cyclic pentamer, and a J or joining

Fab is antigen-binding region

IgM has five subunits

IgA is a monomer or dimer

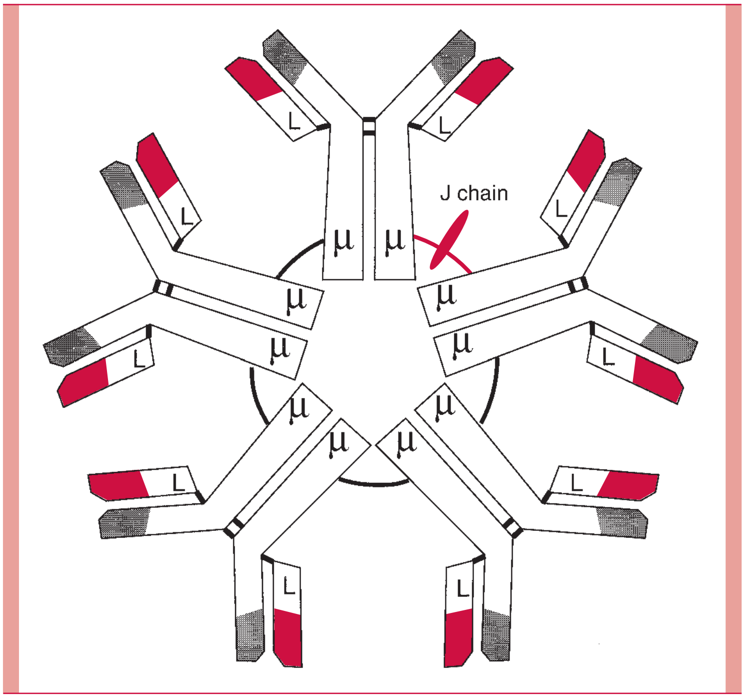


FIGURE 8–6
A planar projection model of serum IgM showing the structure as a cyclic pentamer held together by disulfide bonds. One joining (J) chain is associated with the pentamer. Approximately 10% of the mass of the $\mu$ chain consists of carbohydrate, which is associated with the constant region of the heavy chain.

chain is also associated with the intact structure. When IgM is present on B cells where it serves as a primary receptor for antigen, it is present as a monomer. Other immunoglobulins showing a difference in arrangement from the typical IgG model are the IgA immunoglobulins. In serum these can occur as a monomer, but they can also occur in dimers where the joining chain is required to stabilize the dimer. IgA molecules present in the gut (secretory IgA) occur as dimers where both the J chain and an additional polypeptide, termed the **secretory component,** are present in the complex.

## Functional Properties of Immunoglobulins

### *Immunoglobulin G Antibody*

Immunoglobulin G is the most abundant immunoglobulin in health and provides the most extensive and long-lived antibody response to the various microbial and other antigens that are encountered throughout the life span of the individual. Although at least four subclasses of IgG have been characterized, they are grouped together for the purpose of this chapter. The IgG molecule is bivalent, with two identical and specific combining sites. The rest of the molecule is the constant (Fc) region, which does not vary with differences in specificity of combining sites of different antibody molecules. The constant region has specific sites for binding to phagocytic cells and for reaction with the first component of complement. These sites are made available when the variable region of the antibody molecule has reacted with specific antigen.

Bivalent molecule with specific combining site and constant region

Constant region binds phagocytes

Immunoglobulin G antibody is characteristically formed in large amounts during the secondary response to an antigenic stimulus and usually follows production of IgM (see below) in the course of a viral or bacterial infection. Memory cells are programmed for rapid IgG response when another antigenic stimulus of the same type occurs later. Immunoglobulin G antibodies are the most significant antibody class for neutralizing soluble antigens (eg, exotoxins) and viruses. They act by blocking the sites on the antigenic molecule or virus that determine attachment to cell receptors. IgG also enhances phagocytosis of particulate antigens such as bacteria, because the exposed Fc sites of antibody that is bound to the antigen have a specific affinity for receptors on the surface of phagocytic cells. As described later, the third component of complement also mediates attachment to phagocytes. Enhancement of phagocytosis by antibody, complement, or both is referred to as **opsonization.** Accelerated IgG responses from memory cell expansion frequently

Antibodies produced during secondary response neutralize toxins and viruses

Binding may block attachment receptor

Opsonization enhances phagocytosis

confer lifelong immunity when directed against microbial antigens that are determinants of virulence. There is active transport of the IgG molecule across the placental barrier, which allows maternal protective antibody to pass and, thus, provides passive immune protection to the fetus and newborn pending development of a mature immune system. It is the only immunoglobulin class known to be placentally transferred. The half-life of passively transferred IgG within the same species is approximately 1 month, and thus the infant is protected during a particularly vulnerable period of life.

## *Immunoglobulin M Antibody*

Effective agglutinating antibody

Binds complement at multiple sites

Monomers of IgM constitute the specific epitope recognition sites on B cells that ultimately give rise to plasma cells producing one or another of the different immunoglobulin classes of antibody. Because of its multiple specific combining sites, IgM is particularly effective in agglutinating particles carrying epitopes against which it is directed. It also contains multiple sites for binding the first component of complement. These sites become available once the IgM molecule has reacted with antigen. IgM is particularly active in bringing about complement-mediated cytolytic damage to foreign antigen-bearing cells. It is not, itself, an opsonizing antibody because its Fc portion is not recognized by phagocytes. Opsonization occurs through its activation of the complement pathway; this process is discussed later in the chapter.

Earliest antibody after antigenic stimulation

Does not cross placental barrier

Immunoglobulin M is usually the earliest antibody to appear after an antigenic stimulus, but it tends to decline rapidly and is often succeeded by IgG production from the same clone of cells. It is primarily intravascular and does not cross the placental barrier to the fetus (in contrast to IgG). Thus, the presence of specific IgM against a potentially infecting agent in the blood of a neonate is a priori evidence of active infection rather than of passively acquired antibody from the mother. Antibody response to certain antigens, including the lipopolysaccharide O antigen of Gram-negative bacteria, is characteristically IgM. Some universally occurring antibodies (natural antibodies), such as those directed against blood group antigens, are also of the IgM class.

## *Immunoglobulin A Antibody*

Secretory antibody is produced at mucosal surfaces

Secretory piece combines molecules and resists proteolysis

Immunoglobulin A has a special role as a major determinant of so-called local immunity in protecting epithelial surfaces from colonization and infection. Certain B cells in lymphoid tissues adjacent to or draining surface epithelia of the intestines, respiratory tract, and genitourinary tract are encoded for specific IgA production. After antigenic stimulus, the clone expands locally and some of the IgA-producing cells also migrate to other viscera and secretory glands. At the epithelia, two IgA molecules combine with another protein, termed the **secretory piece,** which is present on the surface of local epithelial cells. The complex, then termed **secretory IgA** (sIgA), passes through the cells into the mucous layer on the epithelial surface or into glandular secretions where it exerts its protective effect. The secretory piece not only mediates secretion but also protects the molecule against proteolysis by enzymes such as those present in the intestinal tract.

Interferes with attachment of microbes to mucosal surfaces

The major role of sIgA is to prevent attachment of antigen-carrying particles to receptors on mucous membrane epithelia. Thus, in the case of bacteria and viruses, it reacts with surface antigens that mediate adhesion and colonization and prevents the establishment of local infection or invasion of the subepithelial tissues. It can agglutinate particles but has no Fc domain for activating the classic complement pathway; however, it can activate the alternative pathway (see below). Reaction of IgA with antigen within the mucous membrane initiates an inflammatory reaction that helps mobilize other immunoglobulin and cellular defenses to the site of invasion. IgA response to an antigen is shorter lived than the IgG response.

## *Immunoglobulin E Antibody*

Bound to mast cells and basophils

Immunoglobulin E is a monomer consisting of two light chains (either $\kappa$ or $\lambda$) and two heavy chains. It is normally present in very small amounts in serum, and most IgE is bound firmly by its Fc portion to tissue mast cells and basophils, which are major producers of histamine. When IgE bound to mast cells reacts with specific antigen, the mast cells degranulate and release histamine and other factors that mediate an inflammatory reaction with dilation of the capillaries, exudation of plasma components, and attraction of neutrophils and eosinophils to the site. Thus, IgE contributes to a rapid second line of defense if surface-protective mechanisms are breached. IgE also plays a significant indirect role in the immune response to a number of helminthic (worm) infections because of attraction of eosinophils to the site at which it reacts with antigen. The eosinophils bind to the Fc portions of IgG molecules that have reacted with surface antigens of the parasite and help bring about its destruction. Certain types of allergies, to be discussed later in this chapter, are due to excessive production of IgE with specificity for a foreign protein. The pharmacologic effects of histamine and the other vasoactive mediators released from mast cells largely account for the symptoms of the disorder.

Important in parasitic infections

Allergies linked to IgE

## *Immunoglobulin D Antibody*

Immunoglobulin D antibody consists of two light chains and two heavy chains. It is highly susceptible to proteolytic enzymes in the tissues and is found only in very low concentrations in serum. Its role is not fully understood, although, as indicated earlier, it is present on the surface of unstimulated B cells and may serve as a receptor for antigen. The chain composition, size, and some major biological properties of the separate classes of immunoglobulins are summarized in Table 8–3.

May be an antigen receptor

TABLE 8–3

**Structural and Biological Properties of Human Immunoglobulins**

| | IgG | IgA | IgM | IgD | IgE |
|---|---|---|---|---|---|
| Heavy chain class | $\gamma$ ($\gamma 1$, $\gamma 2$, $\gamma 3$, $\gamma 4$) | $\alpha$ ($\alpha 1$, $\alpha 2$) | $\mu$ | $\delta$ | $\varepsilon$ |
| Light chain class | $\kappa$ or $\lambda$ | $\kappa$ or $\lambda$ | $\kappa$ or $\lambda$ | $\kappa$ or $\lambda$ | $\kappa$ or $\lambda$ |
| Molecular formula | $\gamma 2\kappa 2$, or $\gamma 2\lambda 2$ | $\alpha 2\kappa 2$ or $\alpha 2\lambda 2$; ($\alpha 2\kappa 2$) SC-J or ($\alpha 2\lambda 2$) SC-J (mucosal form) | $(\mu 2\kappa 2)_5$J or $(\mu 2\lambda 2)_5$J or $\mu 2\kappa 2_m$ or $\mu 2\lambda 2_m$ (B-cell membrane) | $\delta 2\kappa 2_m$ or $\delta 2\lambda 2_m$ | $\varepsilon 2\kappa 2$ or $\varepsilon 2\lambda 2$ |
| Approximate mass | 150,000 | 160,000 400,000 | 900,000 memb. 180,000 | 180,000 | 190,000 |
| Serum concentration (mg/mL) | 10 | 2 | 1.2 | 0.03 | trace |
| Complement fixation (classic) | + | 0 | +++ | 0 | 0 |
| Placental transfer | + | 0 | 0 | 0 | 0 |
| Reaginic activity | ? | 0 | 0 | 0 | +++ |
| Lysis of bacteria | + | + | +++ | ? | ? |
| Antiviral activity | + | +++ | + | ? | ? |
| B-cell receptor for antigen | + (memory) | + (memory) | + (primary) | + (primary) | + (memory) |

## Antibody Production

After a lag phase, the primary response lasts for weeks and then declines

IgM response switches to IgG

Secondary response is primarily IgG

The major events characterizing the general phenomenon of antibody production are illustrated in Figure 8–7 and summarized as follows: Initial contact with a new antigen (primary stimulus) evokes the so-called **primary response,** which is characterized by a lag phase of approximately 1 week between the challenge and the detection of circulating antibodies. In general, the length of the lag phase depends on the immunogenicity of the stimulating antigen and the sensitivity of the detection system for the antibodies produced. **Immunogenicity,** or the capacity to generate an immune response, is contingent on the state of the antigen when injected, the immunologic status of the animal, and the use of adjuvants or nonspecific amplifiers of immune reactivity. Once antibody is detected in serum, the levels rise exponentially to attain a maximal steady state in about 3 weeks. These levels then decline gradually with time if no further antigenic stimulation is given. The major antibodies synthesized in the primary immune response are the immune macroglobulins (IgM class). In the latter phase of the primary response, IgG antibodies arise, and these molecules eventually predominate. This transition is termed the IgM/IgG switch. Following a secondary or booster injection of the same antigen, the lag time between the immunization and the appearance of antibody is shortened, the rate of exponential increase to the maximum steady-state level is more rapid, and the steady-state level itself is higher, representing a larger amount of antibody. Another key factor of the **secondary response** is that the antibodies formed are predominantly of the IgG class. In addition to higher levels of antibody, the secondary IgG antibodies are often better antibodies in the sense that there has been a maturation in affinity of the combining sites so that the secondary antibodies are more effective at binding the antigen than were the IgM and initial IgG molecules produced. This process of affinity maturation results from a process of somatic mutation ongoing during the response.

IgG, IgM, and IgA responses occur during immunization

Quantity may not predict biologic effect

The preceding description of primary and secondary immune responses represents the idealized case that would be expected in normal individuals. Figure 8–8 illustrates the detailed sequence of IgG, IgM, and IgA antibodies to poliovirus that appears in the serum of a child who was immunized with three doses of attenuated live poliovirus. The inactivated virus was given at monthly intervals to a newborn beginning at 2 months of age. The contributions of serum IgM, IgG, and IgA antibodies are individually depicted. The overall capacity of the serum to neutralize the poliovirus is first detectable about 1 week following the primary immunization and reaches a plateau after the second immunization. The IgM antibody peaks at 1 week and gradually declines during the course of vaccination. Primary IgG plateaus at approximately 2 weeks and increases with the secondary booster injection. IgA appears later than either IgM or IgG and is enhanced by secondary and tertiary boosts. It should be emphasized that in developing vaccines, the quantity or

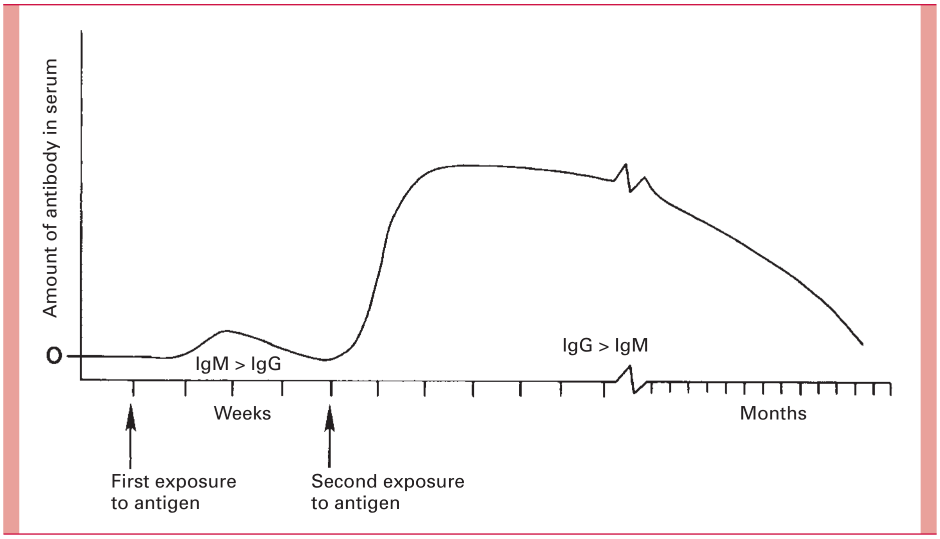


FIGURE 8–7
Primary and secondary immunologic responses. The response to first inoculation of antigen becomes apparent in a week to 10 days. It is small, predominantly of IgM class, and declines rapidly. Activation of memory cells by a second inoculation leads to a much greater, more rapid, and more long-lived IgG response.

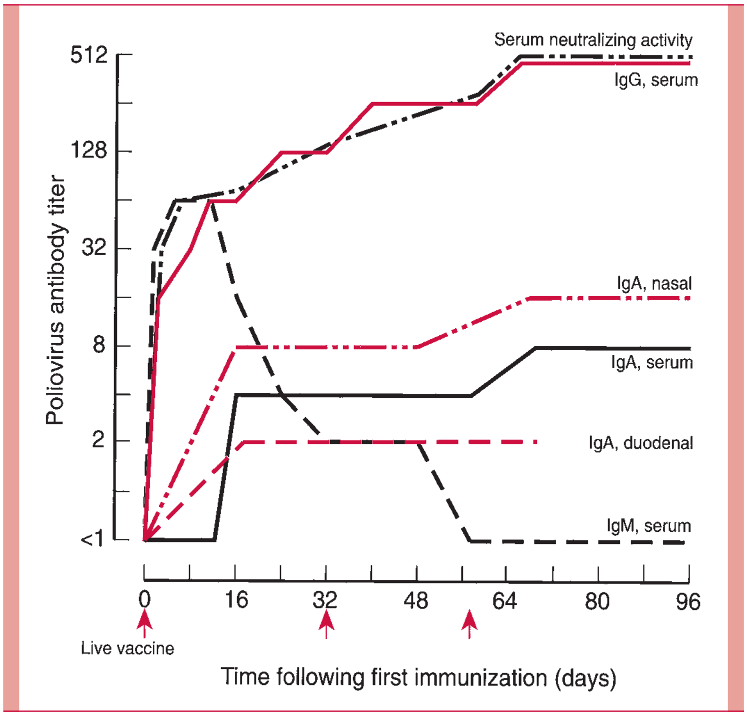


FIGURE 8–8
Detailed sequence of IgG, IgM, and IgA antibodies to poliovirus in serum and secretions of an infant immunized with live attenuated poliovirus. *(Based on Ogra PL, et al.* N Engl J Med *1968;279:893–900.*)

class of antibody produced is secondary to the biological effect. In the polio example, the serum IgA antibody is probably less important than that produced at the mucosal surfaces of the gut where it can block virus attachment.

## Antibody-Mediated Immunity

Antibodies provide immunity to infection and disease in a variety of ways:

1. They can neutralize the infectivity of a virus, the toxicity of an exotoxin molecule, or the ability of a bacterium to colonize. This is usually brought about by reaction between the antibody and an epitope that is required for attachment of the organism or toxin to a target host cell. IgA and IgG antibodies are particularly significant in neutralizing activity.
2. Antibodies can inhibit essential nutrient assimilation by some bacteria. This occurs when a specific antigenic site or protein is involved in transport of the essential nutrient into the cell. For example, some iron-binding siderophores (see Chapter 3) are antigenic, and antibody against them can prevent assimilation of the iron that is essential for growth.
3. Immunoglobulin G antibody can promote phagocytosis of extracellular bacteria by combining with capsules or other surface antigens that otherwise inhibit ingestion of the organism by phagocytes. When antigen–antibody reactions occur, the attachment sites for phagocytes on the Fc regions of the antibodies are exposed, the organism is bound to the phagocyte, and ingestion occurs. The significance of such opsonization is that many bacteria and some viruses are rapidly destroyed within the phagocytic cell.
4. Antigen–antibody reactions involving IgG and IgM activate the classic pathway of the complement cascade, which is described later. Complement components enhance a wide range of nonspecific host defense mechanisms, synergize antibody-mediated opsonization, and lead to lysis of many Gram-negative bacteria with which antibody has reacted. A similar event occurs with blood and tissue cells carrying surface antigens recognized as foreign.
5. Antibodies that recognize foreign antigens on the surface of a host cell, such as a virally infected cell, react with them and can mediate destruction of the cell by the process of antibody-dependent cell-mediated cytotoxicity (ADCC).

Fc portions bind to cells in ADCC

Fab binding of antigen triggers cytotoxicity

In ADCC, the antibodies bind to the cells through their Fc portions that attach to cell surface receptors specific for the Fc regions of particular IgG classes. These are termed Fc receptors (FcR). For example, the human monocyte–macrophage has a plasma membrane receptor that recognizes both IgG1 and IgG3 subclasses through a binding site on the $C_g3$ domain. Eosinophils have a low-affinity FcR for IgE, which is much lower than that of mast cells. If the antibody is bound by its Fc piece, the Fab regions are free to bind antigen to initiate ADCC in the case of monocytes or polymorphs or an allergic response when IgE molecules on mast cells are cross-linked by binding to the antigen (allergen). A variety of clinical problems arise from this antibody-mediated cytotoxicity, including transfusion reactions; autoimmune hemolytic anemias; and the autoimmune disease myasthenia gravis, in which antibodies are directed at the acetylcholine receptor in the motor end plate.

## THE COMPLEMENT SYSTEM

Multiple components reacting in cascade fashion when triggered

No immunologic specificity is involved

Essential for lysis of bacteria by antibody

The complement system plays a critical adjunctive role to the specific immune system. Complement consists of 20 major distinct components and several other precursors. It is a highly complex system, and for the purposes of this chapter, we will focus on only nine major components. Some of the components are proenzymes, and all are present in the plasma of healthy individuals. When the complement system is triggered, a cascade of reactions occurs that activates the different components in a fixed sequence. Several of these activated components have differing and important effects in defense against infection. Components of complement are designated by numbers, which, unfortunately for the student, reflect the order in which they were first described rather than the sequence in which they are activated. There is no immunologic specificity in complement activation or in its effects, although specific antigen–antibody reactions are major initiators of activation, and some complement components enhance the effects of antigen–antibody interactions, for instance, in opsonization.

### Classic Complement Pathway

Antigen antibody reaction exposes complement binding sites

C3b has receptors for phagocytes

The classic complement pathway is summarized in Figure 8–9. It is initiated by antigen–antibody reactions involving IgM or IgG. These reactions expose specific sites on the Fc portion of immunoglobulin molecules that bind and activate the C1 component of complement. C1 then activates C4 and C2, and this complex splits C3 into two components, C3a and C3b. C3a liberates histamine and other vasoactive mediators from mast cells and stimulates the respiratory burst of phagocytes, thus increasing their microbicidal power. C3b binds to the membrane of microorganisms or to such cells as tumor cells or red cells and to specific sites on Fc portions of IgM and IgG. Polymorphonuclear neutrophils (PMNs) and macrophages have receptors for C3b, which thus serves as an opsonin for microorganisms. The opsonic process is markedly enhanced when specific antibody has reacted with the organism.

C5a is chemotactic for PMNs

Complete complex creates membrane holes

C3b, in association with activated C4 and C2, continues the cascade by splitting C5 into two components, C5a and C5b. C5a stimulates release of histamine and other vasoactive mediators from mast cells, is a chemotactic factor for PMNs, and enhances their metabolic antimicrobial activity. C5b binds to the membrane of cells on which an antigen–antibody reaction has occurred and initiates activation of the terminal components C6 to C9. Insertion of the complex C5b, C6, C7, C8, C9 into the cell membrane produces functional holes and leads to the osmotic lysis of eukaryotic cells against which the antibody was directed. Some Gram-negative bacteria are similarly affected when there is an antibody response to accessible sites on the outer membrane. In this case, lysis (bacteriolysis) requires also the activity of lysozyme from phagocytes to break down the peptidoglycan layer of the cell wall.

### Alternative Pathway

Antibody not required for activation

The alternative pathway is more primitive than the classic pathway and does not require the presence of antibody. Instead, C3 can be activated by certain nonimmunologic stimuli. These include endotoxin, other bacterial cell wall components, aggregated IgA, and feedback from activation of the classic pathway. The alternative pathway is shown with the

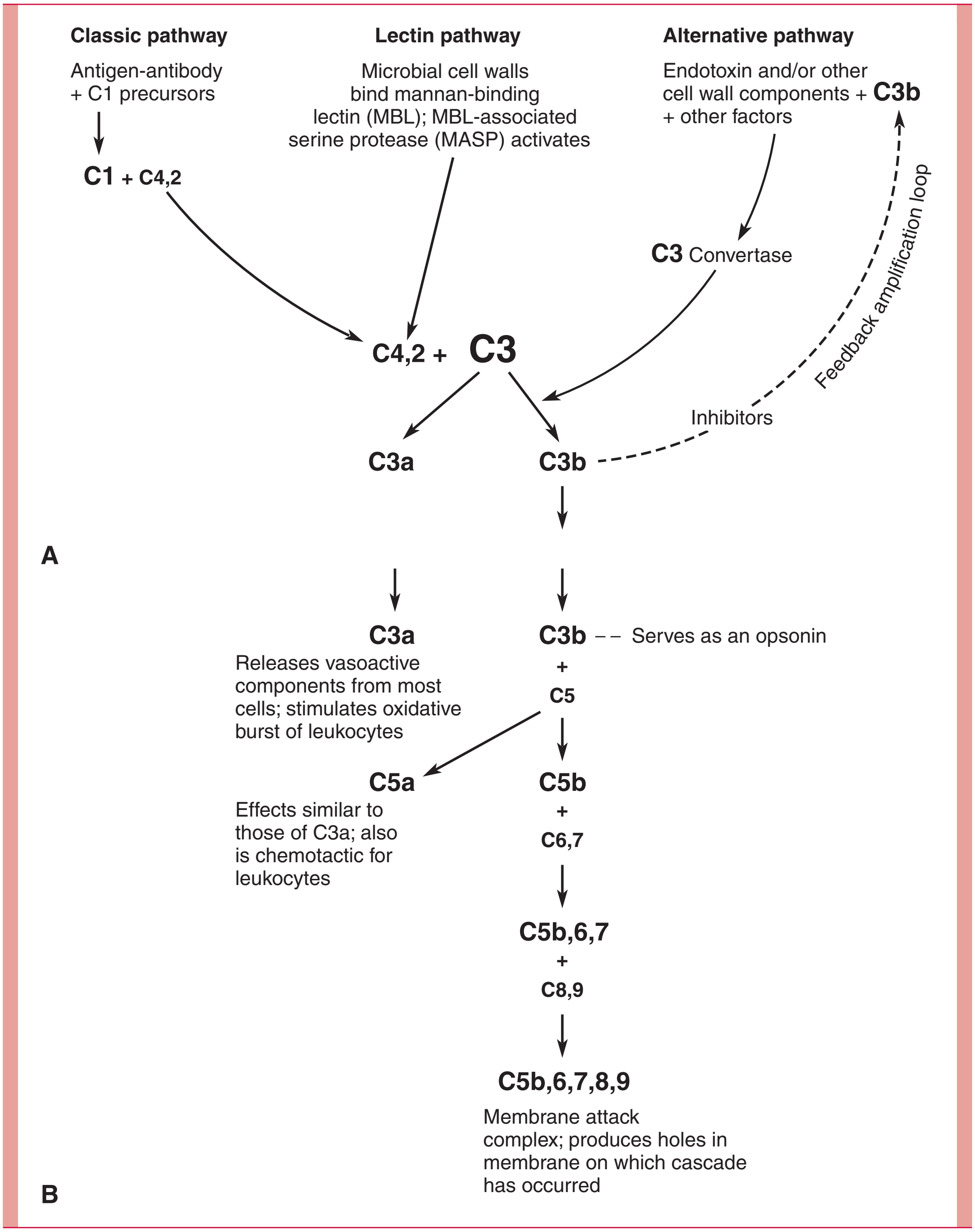


FIGURE 8–9
Schematic of the complement system. **A.** Pathways for activation of C3. (*Note:* Both pathways converge at C3.) **B.** Subsequent cascade and biological effects. Activated components are shown in bold type.

classic pathway in Figure 8–9. It produces the same inflammatory mediators (C3a, C5a) and increased phagocytic activity that result from activation of the classic pathway, but it is not as efficient in cell lysis, because direction of complement components to the cell membrane by antibody is not involved. This pathway is particularly important in early response to infection. Another non–antibody-mediated means of activating the complement system is based on the building of the mannose-binding lectin to pathogens via their carbohydrate-rich external surfaces and activation of serine esterases that act at the level of C4 in the cascade. Inherited deficiencies in complement components are often associated with increased susceptibility to bacterial infections. Most noticeable is the association of recurrent or unusually severe infections due to *Neisseria* (see Chapter 20) and individual complement component deficiencies (usually of C5, C6, C7, or C8).

Important early response

Cell lysis is less efficient

## ADVERSE EFFECTS OF IMMUNOLOGIC REACTIONS AND HYPERSENSITIVITY

Multiple classes of hypersensitivity reactions occur in combinations

Immunologic reactions in the body may result in excessive responses, sometimes far beyond those needed to remove or neutralize microbial pathogens or molecules contributing to disease. Such responses are classified as hypersensitivity reactions if they cause marked physiologic changes or tissue damage or exacerbate disease processes. Four distinct classes of hypersensitivity are recognized, but these do not occur in isolation, and injury often results from a combination of the reactions. In each case they represent an extension of a normal defense mechanism. The immune response in practice is very potent and leads to deleterious consequences for the host if it is in operation too long. The antigen-specific portion of the humoral or T-cell reactions constitutes only a small fraction of the overall response and amplification by complement components that can stimulate phagocytic cells and the release of cytokines; these can cause a general recruitment of lymphocytes, monocytes, and polymorphonuclear cells, leading to a cascade of inflammation and prolonged disease. The aspects of hypersensitivity are overexpressions of the beneficial immune responses that act inappropriately. The normal immune basis of the four types of hypersensitivity are described below and included in Tables 8–1 and 8–4. Types I, II, and III hypersensitivity are mediated by antibody; type IV (delayed-type) hypersensitivity is carried out by antigen-specific T cells assisted by macrophages.

### Anaphylactic (Type I) Hypersensitivity

IgE activities liberate vasoactive mediators from mast cells

Bronchospasm and muscle contraction are life-threatening

Type I hypersensitivity, also called **anaphylaxis,** is represented by the allergic reactions that occur immediately following contact with the sensitizing antigen (allergen). IgE antibodies are bound to Fc receptors on the surface of mast cells (Fig 8–10). If a multivalent antigen binds to the cell-bound IgE molecules, it cross-links them, with the result that the mast cell degranulates, releasing a variety of pharmacologically active mediators. Among the prominent mediators released following binding of the allergen is histamine, which increases capillary permeability and causes bronchoconstriction. Preformed mediators such as the anticoagulant heparin, complement 3 convertase, and a group of compounds involved in chemotaxis of eosinophils, neutrophils, and platelet activation are also released by degranulation. Slow reactive substances involved in bronchoconstriction and

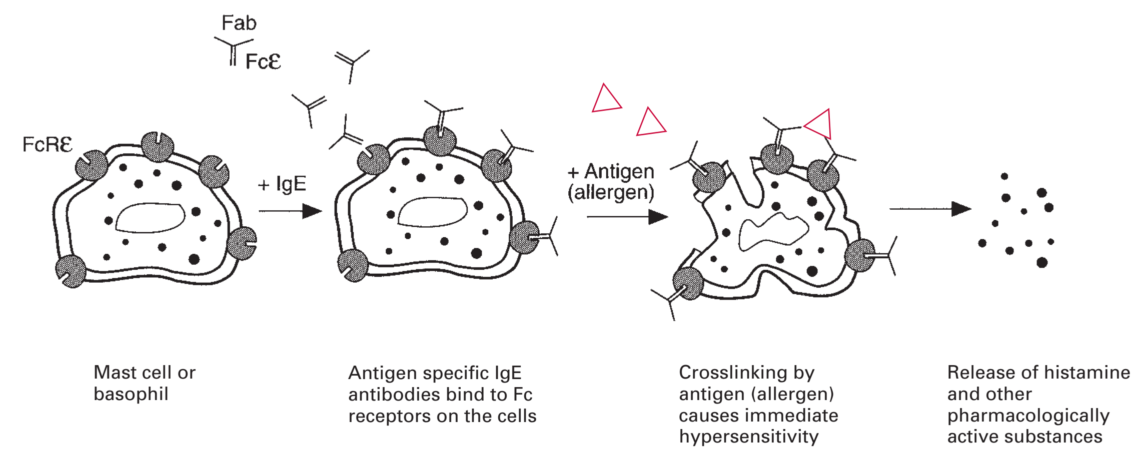


FIGURE 8–10
Diagram outlining the sequence of events in the anaphylactic (allergic) hypersensitive response (type 1) in which IgE antibodies arm mast cells by binding to Fc receptors (FcR) and the response is triggered by cross-linking of these by antigen. Comparable diagrams can be drawn for the arming of macrophages or polymorphonuclear leukocytes by IgG immunoglobulins adhering via their Fc receptors. In these reactions, the Fab arms of the bound antibodies are free to bind antigen specifically, and this binding initiates cellular events leading to sensitized phagocytosis and destruction or, in this case, the release of destructive pharmacologically active substances.

TABLE 8–4

**Effector Cells in Cell-Mediated Immunity**

| Cell | Function | Special Properties |
|---|---|---|
| Specific helper T ($T_H$) cells | MHC class II–restricted help to B cells ($T_H2$), or in activation of macrophages ($T_H1$); distinct cytokines are used in the two processes | $T_H$ cells use $\alpha/\beta$ Tcr; $T_H2$ cells can activate eosinophils as well as B cells through IL-5; $T_H1$ cells can activate NK cells through IL-12, and macrophages through IL-2 and IFN-$\gamma$, $T_H2$ cells can communicate either positively or negatively with one another via cytokines |
| Specific cytotoxic T ($T_C$) cells | MHC class I–restricted specific killing; use endogenous $\alpha/\beta$ Tcr | Can kill multiple targets sequentially; have major role in eliminating virally infected target cells |
| Specific suppressor T ($T_S$) cells | Antigen specific; involves "suppressor inducer" T cells (CD4), which generate "suppressor effector"; $T_S$ cells can be antigen specific or idiotype specific; some aspects of the interactions are MHC restricted | "Infectious tolerance" (ie, transfer of $T_S$ cells transfers antigen-specific immunosuppression) |
| Regulatory T cells | Antigen specific | CD4+, CD25+, suppress inflammation |
| "K cells," macrophages, polymorphonuclear cells | Antibody-dependent cell-mediated cytotoxicity (ADCC) | Bind IgG via Fc receptors; IgG acts as an antigen-specific opsonin on these cells |
| Natural killer (NK) cells | Occur in unchallenged animals; can kill a variety of tumors and virus-infected or embryonic cells in vitro without expression of classic Tcr or bound antibody | Are large, granular lymphocytes; do not phagocytize target cells but kill by release of toxins |
| NK T cells | Tcr $\alpha/\beta$ but restricted V$\alpha$ | CD4+ T cells produce cytokines that stimulate $T_H1$ (IF-$\alpha$) and $T_H2$ (IL-4) responses |

chemotaxis are also produced, as are prostaglandins and thromboxanes, which are implicated in bronchospasm, muscle contraction, and platelet aggregation. Thus, the specific binding of an allergen to the combining site of its antibody can result in a potent release of compounds, leading to painful and life-threatening consequences for the allergic individual. Despite the suffering that hypersensitivity to common allergens such as pollen, bee stings, house dust, and cat dander brings to a large percentage of people, there are possible beneficial consequences of binding of allergen by IgE. These include situations in which ADCC by monocytes and eosinophils may provide protection against parasites such as schistosomes (a trematode worm) and trypanosomes (a protozoan).

When hypersensitivity is very marked, or antigen is introduced systemically, mast cells throughout the body degranulate, and systemic anaphylaxis results with constriction of the bronchi, edema of the larynx and other tissues, vascular collapse, and sometimes death. A generalized anaphylactic reaction rarely if ever occurs as a manifestation of an infection but may occur following parenteral inoculation of an antigen to which the individual has been sensitized (eg, bee sting venom). It may also occur in individuals who have been sensitized to a low-molecular-weight hapten that binds to a tissue protein and becomes antigenic because of the size of the complex. An IgE response to hapten epitopes can then lead to anaphylactic-type hypersensitivity if the epitope is again encountered. A penicillin degradation product has this property, and occasionally, individuals develop severe anaphylactic reactions to penicillins, although this complication is very rare.

Mast cells throughout the body may degranulate

Systemic anaphylaxis may occur with low-molecular-weight haptens

Rapid therapeutic intervention is critical in systemic anaphylaxis. It includes parenteral administration of epinephrine, which reverses the major manifestations of the syndrome by producing bronchodilation, vasoconstriction, and increased blood pressure. Tracheostomy or intubation may be needed to overcome respiratory obstruction due to laryngeal edema.

Epinephrine reverses anaphylaxis

## Antibody-Mediated (Type II) Hypersensitivity

Type II hypersensitivity is an inappropriate elaboration of antibody-dependent cytotoxicity that occurs when antibody binds to antigens on host cells, leading to phagocytosis, killer cell activity, or complement-mediated lysis. Antibody directed against cell surface or tissue antigens results in the fixation of complement such that a variety of effector cells become involved. The cells to which the antibody is specifically bound, as well as the surrounding tissues, are damaged because of the inflammatory amplification. Such mechanisms appear to be responsible for the tissue damage of rheumatic fever following a streptococcal infection or some clinical manifestations of viral diseases, such as group B coxsackievirus infection (see Chapter 36). These phenomena may involve not only antibodies but also cytotoxic T cells. It should be recalled that humoral antibodies are required to arm macrophages, and PMNs are needed to bind cellular antigens in ADCC and to serve as opsonins that facilitate ingestion with eventual intracellular destruction of target cells by macrophages.

Host cells are damaged when targeted antigen is bound to cell surface

Reactions may follow infections

## Immune Complex (Type III) Hypersensitivity

When IgG is mixed in appropriate proportions with multivalent antigen molecules (ie, bearing multiple epitopes), aggregates containing a lattice of many antigen and antibody molecules forms. With appropriate concentrations of the two reactants, a macroscopic precipitate can develop (see Chapter 15). A similar situation applies to IgM, which is multivalent. When the epitope is present on the surface of a larger particle, such as a bacterium or red blood cell, the particles can be cross-linked by antibody, and microscopic or macroscopic agglutination results. These phenomena can occur in vivo when sufficient amounts of specific antibody and of free antigen from an infecting microorganism react locally or in the bloodstream to form an antigen–antibody lattice; the size of the immune complex depends on the relative properties of the two reactants. Large immune complexes are phagocytosed and usually broken down within the phagocyte. However, smaller complexes are deposited in small blood vessels and capillaries through which they do not pass, activate the complement system, and thus produce an acute inflammatory response mediated largely by C3a and C5a. This results in the manifestations of vasculitis. Phagocytes attracted chemotactically to the site release hydrolytic enzymes, and the sum of these effects is acute tissue damage, which can become chronic depending on the survival of the antigen or on whether it is continually replaced. Acute glomerulonephritis following certain streptococcal infections is an example of an immune complex disease in which glomeruli of the kidney are damaged by the complexes, resulting in various manifestations of renal impairment. Inflammatory skin lesions can result from deposition of immune complexes in the cutaneous blood vessels in patients with infective endocarditis. Deposition in joints, the pericardium, or the pleura produces arthritis, pericarditis, and pleuritis or pleurisy, respectively.

Cross-linking forms lattice of antigen and antibody

Small complexes reach capillaries

Complement deposition attracts phagocytes

A systemic form of immune complex disease, termed **serum sickness,** can follow the injection of foreign antigen. An example is the therapeutic use of diphtheria antitoxin that has been produced in horses. About 10 days after inoculation, sufficient antibody against

Antitoxins can form immune complexes

horse proteins has been produced to form immune complexes made up of human antibody reacting against horse serum protein (including horse immunoglobulins). These complexes are deposited in various organs, resulting in a syndrome of arthritis, nephritis, rash, urticaria, and fever. The disease usually resolves as the foreign antigen concentration decreases through immune clearance and catabolism of the antigen(s).

### Delayed-Type (Type IV) Hypersensitivity

The fourth type of hypersensitivity is termed, **delayed-type hypersensitivity.** Unlike types I, II, and III, this process cannot be transferred from one animal to another by serum alone. However, it can be transferred by antigen-specific T lymphocytes. All are initiated by the function of antigen-specific T cells, which then recruit effector cells into the area of recognition of the antigen. Unlike the forms of "immediate" hypersensitivity that can be transferred by antibody, delayed-type hypersensitivity requires days to weeks to express full reactivity. In all of these, the initial reaction is the induction and function of antigen-specific T cells that bear $\alpha/\beta$ T-cell receptors and have been generated in response to antigenic challenge. The time course depends on the involvement of other cells and the properties of the infectious agent involved.

Requires T lymphocyte transfer

Four major types of delayed-type hypersensitivity are all part of the same process differing in site, mechanism of challenge, and timing. The shortest is the Jones–Mote phenomenon, in which the site of antigen injection is infiltrated by basophils, and the skin swelling is maximal 24 hours after antigen injection. This type of hypersensitivity can be raised to soluble antigens, and the reactivity disappears following the appearance of antibody. Contact and tuberculin-type hypersensitivity show maximal reactivity at 48 to 72 hours. Contact sensitivity is observed in response to sensitization with common antigens such as chemicals found in rubber or the small organic compounds produced by poison ivy and poison oak. It is predominantly an epidermal reaction, in contrast to the tuberculin-type hypersensitivity, which is a dermal reaction. The cell that presents antigen for contact sensitization is the Langerhans cell, a dendritic antigen-presenting cell carrying MHC class II antigens. Tuberculin-type hypersensitivity is manifested by individuals who have been sensitized with lipoprotein antigens derived from the tubercle bacillus. Twenty-four hours after intradermal injection of tuberculin, an antigen derived from *Mycobacterium tuberculosis,* there is intense infiltration by lymphocytes, which reaches a maximum in 2 to 3 days.

Contact sensitivity is from chemicals

Tuberculin hypersensitivity is from sensitization by lipoprotein from tubercle bacillus

Probably the most clinically important form of type IV sensitivity is the granuloma, an organized inflammatory lesion that requires at least 14 days to develop. These result from the long-term continuation of the stimulation of effector cells by cytokines produced in initial antigen-specific T-cell response. Granulomatous lesions are a major part of the disease process in chronic diseases caused by bacteria (tuberculosis), fungi (histoplasmosis), and parasites (schistosomiasis).

Long-term stimulation is required for granuloma

## TOLERANCE

As discussed earlier, induced cellular and antibody responses follow challenge with antigens that are normally foreign; however, immunization may not only induce the enhanced reactivities described but may also lead to a diminished reactivity known as **tolerance.** When specifically diminished reactivity is induced by treatment with large doses of antigen, the phenomenon is referred to as **immune paralysis.** Because the immune system is based on a random generation of combining sites directed against molecular configurations, there is in principle no reason why the immune response cannot react with self components. When it does, autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and others may result. However, it is now known that normal healthy individuals express detectable levels of autoantibodies against a variety of self components. A regulatory function for these autoantibodies in the maintenance of homeostasis is suggested by the fact that aged red cells are removed from human circulation by a natural mechanism in which normally occurring IgG autoantibodies specific for a modified membrane component (senescent cell antigen) bind to the cells, leading to their

Diminished reactivity prevents pathologic reactivity to self antigens

Autoantibodies may have homeostatic functions removal by phagocytic cells. Nevertheless, the generation of tolerance or the inability to react against self is a fundamental part of the process of development in vertebrates, which results essentially from the removal or inactivation of T cells in the thymus that can react to self antigens.

B-cell tolerance also occurs

Parallel tolerization procedures for B cells occur, but currently a number of mechanisms now must be proposed for maintenance of nonreactivity to self. Antigen-specific T cells may be either deleted by contact with antigen (clonal abortion) or inactivated without being destroyed. In addition, suppressor T cells that downregulate the specific immune response may be generated that either shut off the antigen-specific helper T cells or are directed toward combining sites of B-cell antibodies. Antigen-specific B cells may be deleted or inactivated or rendered insensitive to secondary stimulation by cytokines. These central effects operate at the level of antigen-specific T or B cells.

Tolerance may be disturbed by quantitative or cross-reactive mechanisms

Both experimentally induced tolerance and innate tolerance can be broken down in two general ways. First, if a large amount of antigen is needed to maintain tolerance, immunity can be generated if the level of antigen falls below the required tolerogenic level. A second way of breaking of tolerance is immunization with a cross-reactive antigen. Two clinically well-known examples of the capacity of cross-reactive antigens to break normal self tolerance are (1) the induction of experimental allergic encephalomyelitis in an animal by the injection of heterologous brain tissue homogenates in emulsified adjuvant and (2) the capacity of infections with group A streptococci to cause rheumatic fever because of a cross-reaction between bacterial antigens and myocardial tissue. The concept of tolerance is critical to much of modem medicine because of increasing interest in autoimmune diseases, which can be considered to result from a failure or breakdown of tolerance.

## FUNCTIONAL INTEGRATION OF THE IMMUNE SYSTEM IN RESPONSE TO INFECTIOUS ORGANISMS

Viral proteins induce $T_H1$ responses and cytokines

B cells and macrophages are activated later

$T_H1$ is effective against intracellular parasites

The innate immune system involves phagocytic cells such as monocytes, macrophages and dendritic cells, and cytokines such as interleukin-1 that are generated following activation of phagocytic cells by binding of bacterial lipopolysaccharide to surface receptors. Once the innate system is activated, the cytokines it produces and the peptide antigens presented to T cells serve to activate and condition the response of the specific adaptive system (Fig 8–11). The upper half of the figure illustrates how activation of the antigen-presenting cell (monocyte, macrophage, or dendritic cell) can stimulate NK cells, CD8+ cytotoxic T cells, or $T_H1$ type CD4+ cells. The $T_H1$ cells are induced by presentation of peptides derived from antigens such as a viral coat protein presented to the $\alpha/\beta$ T-cell receptor of an unstimulated T cell with the activation and transformation process mediated by IL-12 and IFN-$\alpha$. Once the $T_H1$ cell is specifically activated, the process can lead to the activation of B cells to make IgM or IgG but, more importantly, to activate macrophages to act in an inflammatory manner. $T_H1$ type cell-mediated immunity is particularly effective against intracellular parasites but has the drawback of increasing the severity of autoimmune diseases.

$T_H2$ responses activate B cells and immunoglobulins

Protective against helminth worm infections

The lower half of (Figure 8–11) illustrates the activation of $T_H2$ type helper cells via the mediation of the cytokine IL-6 and the production of IL-4 to drive the differentiation pathway. The specificity for antigen is maintained by presentation of peptide antigen via MHC of the antigen presenting cell to the $\alpha/\beta$ T-cell receptor of the unstimulated helper T cell. A separate type of NK cell, one that is CD4+ and expresses a restricted Tcr V$\alpha$ is involved in this process. $T_H2$ type immunity is most prominent in the activation of B cells, allowing the generating of IgM, IgG, IgA, and IgE. Antibodies are valuable in the protective immune response to many bacteria and also in maintaining protection against viruses. Most notably, the $T_H2$ response is host protective in infections by gastrointestinal helminth worms such as schistostomes, where production of specific IgE antibody bound to macrophages, basophils, or acinophils appears to confer protection. On the other hand, $T_H2$ type immunity in antibodies appear to offer little protection against retroviruses, including HIV. IgE production to allergens produced by dust mites or ragweed may lead to serious clinical consequences of allergic responses.

The above scheme depicting the critical role of the polarization of helper T cell type and function in resistance to certain diseases and in the exacerbation of others is not yet

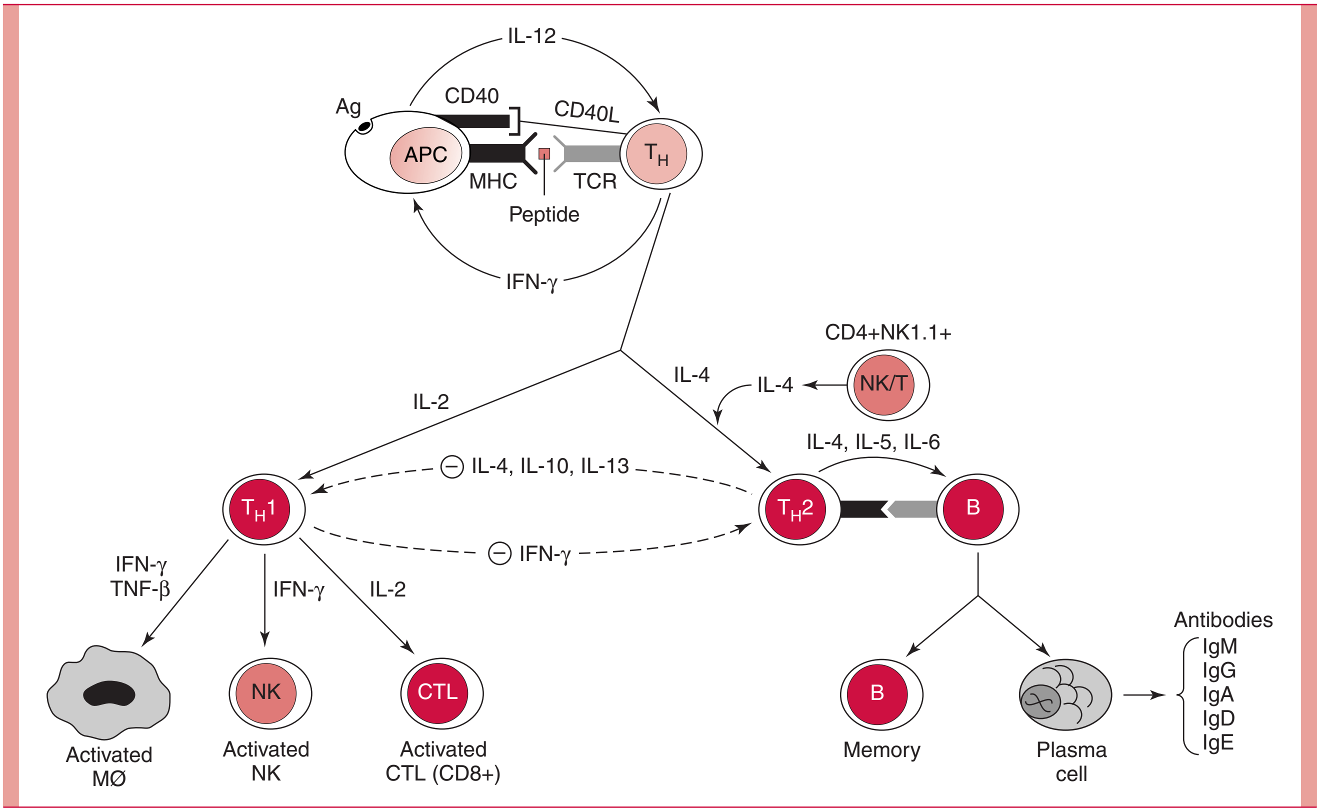


FIGURE 8–11

Diagram of $T_H1$ and $T_H2$ cells in the generation of cell-mediated immunity or antibody production. *Abbreviations*: AG, antigen; APC, antigen-presenting cell; B, B cells; CTL, cytotoxic T lymphocyte; CD, cell surface determinant; IFN, interferons; IL, interleukins; MHC, major histocompatibility complex; MΦ, macrophage; NK, natural killer cell; NK/T, natural killer cell related to T cells; TCR, T cell receptor; $T_H$, helper T cells.

completely established. It has been sufficiently documented to make it a worthwhile overall conceptual framework in which to place infectious diseases caused by distinct types of pathogens, autoimmune diseases, immunity to tumors, and allergy. The difficulty is that there is no such thing as a pure $T_H1$ or $T_H2$ response; rather, there is a balance between the two types of effector T cells as manifested by levels of cytokines. In the most simple case, bacteria coated with polysaccharides that protect them from ingestion by phagocytes are readily attacked by antibodies. Furthermore, antibodies of the IgM class against these polysaccharides can be generated in the relative absence of T-cell help. Nonetheless, $T_H2$ type cytokines are required for activation and differentiation of the B cells and their differentiation into antibody-secreting plasma cells. Recently, it has also been shown that natural antibodies to viruses are protective in experimental infections of mice. *S. pneumoniae* are extracellular pathogens that enter the lungs and colonize the space in the alveoli, where their multiplication causes tissue damage and inflammation that can impair breathing. Antibodies to these organisms enable them to be phagocytized and also to be killed by activation of the complement cascade following binding of the antibody. By contrast, *Leishmania* is an intracellular parasite that proliferates within macrophages inside vesicles called endosomes. Thus, the parasites are protected from attack by antibodies. $T_H1$ type immunity plays a major role in their destruction because the infected macrophages can break down the organisms into peptides that are then presented by MHC class II molecules to the receptor on CD4+ cells. The T cells then become activated by interaction of the accessory CD28 molecule on the T cell with the B7 molecule on the macrophage. The activated $T_H1$ type cells now secrete cytokines such as IFN-$\gamma$ that induce the macrophage to produce tumor necrosis factor and nitric oxide that kill the parasites within the cells. Viruses are intracellular parasites that replicate within the nucleus or within the cytoplasm. Both the

$T_H1$ and $T_H2$ responses are balanced, not pure

Antipolysaccharide antibodies facilitate complement deposition and phagocytosis

$T_H1$-stimulated cytokines cause intracellular killing production of cytotoxic CD8+ T cells and $T_H1$ type inflammation are protective against virus infections. Cytotoxic T cells are induced by the presentation of viral peptides by MHC class I molecules, as opposed to helper cell reactivity, that involves that presentation of antigenic class II MHC.

Strong cell-mediated immunity and $T_H1$ in leprosy lead to resolution

$T_H2$ responses are associated with bacterial proliferation

**Leprosy** appears to be a human disease for which the elaboration of a $T_H1$ type response is essential for cure, but the elaboration of $T_H2$ type responses is harmful to the infected person. There are two polar forms of clinical presentation of leprosy. Tuberculoid leprosy is characterized by a strong cell-mediated immune response to the causative organism, *Mycobacterium leprae*. This cell-mediated $T_H1$ type immune response kills the mycobacteria but at the price of immune-mediated tissue damage to the host. Lepromatous leprosy, the other extreme of the clinical spectrum, is characterized by a pronounced antibody response in the virtual absence of a cellular response against the bacterial pathogen. This situation results in extensive bacterial loads and ultimately in the death of the patient. Analyses of messenger RNA in lesions of the two types of leprosy indicate that $T_H1$ type cytokines predominate in the tuberculoid form and $T_H2$ cytokines are the major types generated in lepromatous leprosy. In parallel, $T_H1$ immunity is more effective in the response to *Mycobacterium tuberculosis* than are antibodies.

$T_H2$ responses clear intestinal worms

A vigorous $T_H2$ type response is required for the clearance of infections with gastrointestinal helminths. There is convincing evidence that $T_H2$ type responses are required for the explusion of gastrointestinal parasites, but the exact mechanisms by which the $T_H2$ type cells mediate the protective responses are unknown. The cytokine IL-4 induces the production of IgG1 (in mice) and IgE, generation of mast cells in the intestinal mucosa, increased contractility of the intestine smooth musculature, and reduced intestinal fluid uptake. IL-5 is induced by infection with intestinal nematodes (roundworms), and this cytokine promotes the production and activation of eosinophils. Although the production of IgE and its binding to mast cells and basophils in producing allergic responses is generally considered destructive, recent evidence suggests that important eosinophils and these allergy-type reactions may be mediating immunity against extraintestinal helminth larvae, including those of schistomes (parasitic flatworms).

## Antigen Surrogates

Antibody protein can induce antibodies directed against Fab and epitope recognition sites

A recently developed approach has the potential to allow the use of antibodies as antigen surrogates in immunization. Antibodies are themselves antigenic in animal species to which they are foreign. The antiantibodies that can be produced include some with specificity for unique epitope-reacting portions of the Fab variable region of the antibody against which they are directed as well as for epitope-recognizing sites on immunoresponsive B cells. These are termed **anti-idiotypic antibodies** and have the same three-dimensional geometry as would the epitope molecule. Monoclonal antibodies that have this structure can be selected and produced in large amounts and may then act as antigens for the production of specific antibodies against the epitope of interest. Immunization with such anti-idiotype antibodies has promise for producing specific immunity against critical antigens that are impossible or uneconomical to produce in bulk. At present, there are many problems to overcome before such procedures could begin to be applied to humans.

## ADDITIONAL READING

Abbas AK, Lichtman AH, Pober JS. *Cellular and Molecular Immunology*. Philadelphia: WB Saunders; 2000. A detailed survey of immunologic mechanisms.

Eisen HN. *General Immunology*. Philadelphia: JB Lippincott; 1990. A comprehensive presentation of cellular and molecular immunologic mechanisms.

Janeway CA, Travers P, Walport M, Capra JD. *Immunobiology,* 4th ed. New York: Current Biology Publications; 1999. A detailed survey of contemporary immunology.

Paul WE. *Fundamental Immunology,* 4th ed. New York: Lippincott-Raven Press; 1999. A comprehensive, detailed, multiauthored volume.

CHAPTER 9

# Normal Microbial Flora

KENNETH J. RYAN

The term **normal flora** is used to describe microorganisms that are frequently found in various body sites in normal, healthy individuals. The constituents and numbers of the flora vary in different areas and sometimes at different ages and physiologic states. They comprise microorganisms whose morphologic, physiologic, and genetic properties allow them to colonize and multiply under the conditions that exist in particular sites, to coexist with other colonizing organisms, and to inhibit competing intruders. Thus, each accessible area of the body presents a particular ecologic niche, colonization of which requires a particular set of properties of the invading microbe. The number of organisms in the flora is estimated to exceed the number of cells in the body by a factor of 10.

Organisms of the normal flora may have a symbiotic relationship that benefits the host or may simply live as commensals with a neutral relationship to the host. A parasitic relationship that injures the host would not be considered "normal," but in most instances not enough is known about the organism–host interactions to make such distinctions. Like houseguests, the members of the normal flora may stay for highly variable periods. **Residents** are strains that have an established niche at one of the many body sites, which they occupy indefinitely. **Transients** are acquired from the environment and establish themselves briefly but tend to be excluded by competition from residents or by the host's innate or immune defense mechanisms. The term **carrier state** is used when potentially pathogenic organisms are involved, although its implication of risk is not always justified. For example, *Streptococcus pneumoniae,* a cause of pneumonia, and *Neisseria meningitidis,* a cause of meningitis, may be isolated from the throat of 5 to 40% of healthy people. Whether these bacteria represent transient flora, resident flora, or carrier state is largely semantic. The possibility that their presence could be the prelude to disease is impossible to determine simply by culture of a normal flora site.

Flora may stay for short or extended periods

If pathogens are involved the relationship is called the carrier state

It is important for students of medical microbiology and infectious disease to understand the role of the normal flora, because of its significance both as a defense mechanism against infection and as a source of potentially pathogenic organisms. English poet W. H. Auden understood the desired state of balance between host and microbial flora when he wrote:

Balance is the desired state

Build colonies: I will supply
  adequate warmth and moisture,
the sebum and lipids you need,
  on condition you never
do me annoy with your presence,
  but behave as good guests should,
not rioting into acne
  or athlete's-foot or a boil.

FROM AUDEN WH,
*Epistle to a Godson*

It is also important to know its sites and composition to avoid interpretive confusion between normal flora species and pathogens when interpreting laboratory culture results.

## ORIGIN OF THE NORMAL FLORA

Initial flora is acquired during and immediately after birth

The healthy fetus is sterile until the birth membranes rupture. During and after birth, the infant is exposed to the flora of the mother's genital tract, to the skin and respiratory flora of those handling it, and to organisms in the environment. During the infant's first few days of life, the flora reflects chance exposure to organisms that can colonize particular sites in the absence of competitors. Subsequently, as the infant is exposed to a broader range of organisms, those best adapted to colonize particular sites become predominant. Thereafter, the flora generally resembles that of other individuals in the same age group and cultural milieu.

## FACTORS DETERMINING THE NATURE OF THE NORMAL FLORA

Physiologic conditions such as local pH influence colonization

Adherence factors counteract mechanical flushing

Ability to compete for nutrients is an advantage

Local physiologic and ecologic conditions determine the nature of the flora. These conditions are sometimes highly complex, differing from site to site, and sometimes vary with age. Conditions include the amounts and types of nutrients available, pH, oxidation–reduction potentials, and resistance to local antibacterial substances such as bile and lysozyme. Many bacteria have adhesin-mediated affinity for receptors on specific types of epithelial cells, which facilitates colonization and multiplication while avoiding removal by the flushing effects of surface fluids and peristalsis. Various microbial interactions also determine their relative prevalence in the flora. These interactions include competition for nutrients, inhibition by the metabolic products of other organisms (eg, by hydrogen peroxide or volatile fatty acids), and production of antibiotics and bacteriocins.

## NORMAL FLORA AT DIFFERENT SITES

The total normal flora of the body probably contains more than 1000 distinct species of microorganisms. The major members known to be important in preventing or causing disease as well as those that may be confused with etiologic agents of local infections are summarized in Table 9–1, and most are described in greater detail in subsequent chapters. The student should not attempt to memorize unfamiliar names at this point.

### Blood, Body Fluids, and Tissues

Tissues and body fluids such as blood are sterile in health

Transient bacteremia can result from trauma

In health, the blood, body fluids, and tissues are sterile. Occasional organisms may be displaced across epithelial barriers as a result of trauma (including physiologic trauma such as heavy chewing) or during childbirth; they may be briefly recoverable from the bloodstream before they are filtered out in the pulmonary capillaries or removed by cells of the reticuloendothelial system. Such transient bacteremia may be the source of infection when structures such as damaged heart valves and foreign bodies (prostheses) are in the bloodstream.

### Skin

Propionibacteria and staphylococci are dominant bacteria

Skin flora is not easily removed

Conjunctiva resembles skin

The skin plays host to an abundant flora that varies somewhat according to the number and activity of sebaceous and sweat glands. The flora is most abundant on moist skin areas (axillae, perineum, and between toes). Staphylococci and members of the genus *Propionibacterium* occur all over the skin, and facultative diphtheroids (corynebacteria) are found in moist areas. Propionibacteria are slim, anaerobic, or microaerophilic Gram-positive rods that grow in subsurface sebum and break down skin lipids to fatty acids. Thus, they are most numerous in the ducts of hair follicles and of the sebaceous glands that drain into them. Even with antiseptic scrubbing it is difficult to eliminate bacteria from skin sites, particularly those bearing pilosebaceous units. Organisms of the skin flora are resistant to the bactericidal effects of skin lipids and fatty acids, which inhibit or kill many extraneous bacteria. The conjunctivae have a very scanty flora derived from the skin flora. The low bacterial count is maintained by the high lysozyme content of lachrymal secretions and by the flushing effect of tears.

TABLE 9–1

**Predominant and Potentially Pathogenic Flora of Various Body Sites**

| | Flora | |
|---|---|---|
| Body Site | Potential Pathogens (Carrier) | Low Virulence (Resident) |
| Blood | None | None[a] |
| Tissues | None | None |
| Skin | *Staphylococcus aureus* | *Propionibacterium, Corynebacterium* (diphtheroids), coagulase-negative staphylococci |
| Mouth | *Candida albicans* | *Neisseria* spp., viridans streptococci, *Moraxella, Peptostreptococcus* |
| Nasopharynx | *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae,* group A streptococci, *Staphylococcus aureus* (anterior nares) | *Neisseria* spp., viridans streptococci, *Moraxella, Peptostreptococcus* |
| Stomach | None | Streptococci, *Peptostreptococcus,* others from mouth |
| Small intestine | None | Scanty, variable |
| Colon | | |
| Breastfeeding infant | None | *Bifidobacterium, Lactobacillus* |
| Adult | *Bacteroides fragilis, Escherichia coli, Pseudomonas, Candida, Clostridium (C. perfringens, C. difficile)* | *Bifidobacterium, Lactobacillus, Bacteroides, Fusobacterium,* Enterobacteriaceae, *Enterococcus, Clostridium* |
| Vagina | | |
| Prepubertal and Postmenopausal | *C. albicans* | Diphtheroids, staphylococci, Enterobacteriaceae |
| Childbearing | Group B streptococci, *C. albicans* | *Lactobacillus,* streptococci |

[a]Organisms such as viridans streptococci may be transiently present following disruption of a mucosal site.

## Intestinal Tract

The **mouth** and **pharynx** contain large numbers of facultative and strict anaerobes. Different species of streptococci predominate on the buccal and tongue mucosa because of different specific adherence characteristics. Gram-negative diplococci of the genera *Neisseria* and *Moraxella* make up the balance of the most commonly isolated facultative organisms. Strict anaerobes and microaerophilic organisms of the oral cavity have their niches in the depths of the gingival crevices surrounding the teeth and in sites such as tonsillar crypts, where anaerobic conditions can develop readily. Anaerobic members of the normal flora are major contributors to the etiology of dental caries and periodontal disease (see Chapter 62).

Oropharynx has streptococci and *Neisseria*

The total number of organisms in the oral cavity is very high, and it varies from site to site. Saliva usually contains a mixed flora of about $10^8$ organisms per milliliter, derived mostly from the various epithelial colonization sites. The stomach contains few, if any, resident organisms in health because of the lethal action of gastric hydrochloric acid and peptic enzymes on bacteria. The small intestine has a scanty resident flora, except in the lower ileum, where it begins to resemble that of the colon.

Stomach and small bowel have few residents

Small intestinal flora is scanty but increases toward lower ileum

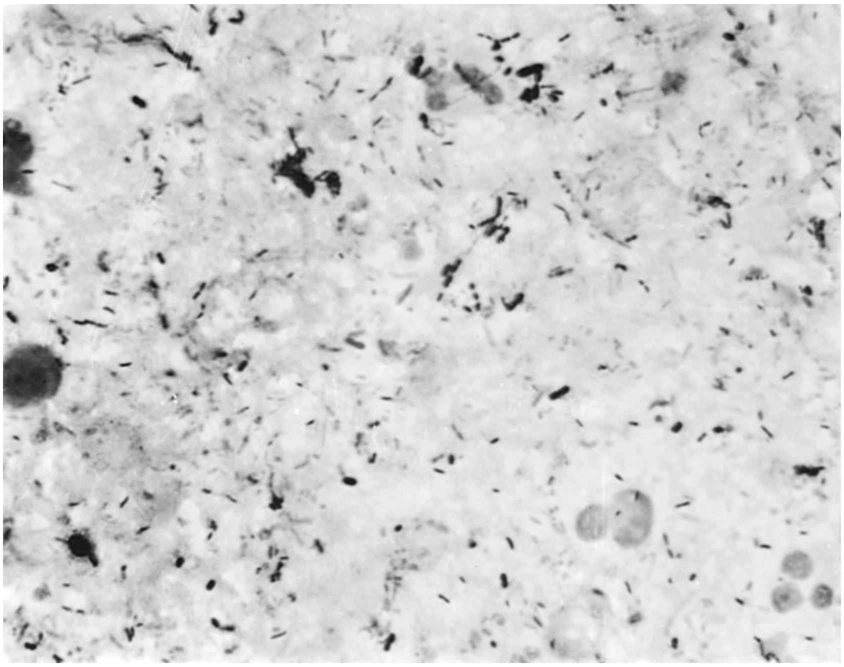

FIGURE 9–1
Smear of feces, showing great diversity of microorganisms.

Adult colonic flora is abundant and predominantly anaerobic

Diet affects species composition

The colon carries the most prolific flora in the body (Fig 9–1). In the adult, feces are 25% or more bacteria by weight (about $10^{10}$ organisms per gram). More than 90% are anaerobes, predominantly members of the genera *Bacteroides, Fusobacterium, Bifidobacterium,* and *Clostridium*. The remainder of the flora is composed of facultative organisms such as *Escherichia coli,* enterococci, yeasts, and numerous other species. There are considerable differences in adult flora depending on the diet of the host. Those whose diets include substantial amounts of meat have more *Bacteroides* and other anaerobic Gram-negative rods in their stools than those on a predominantly vegetable or fish diet.

Bifidobacteria are predominant flora of breastfed infants

Bottle-fed infants have a flora similar to that of weaned infants

The fecal flora of breastfed infants differs from that of adults, with anaerobic Gram-positive rods of the genus *Bifidobacterium* constituting as much as 99% of the total. Human milk is high in lactose and low in protein and phosphate, and its buffering capacity is poor compared with that of cow's milk. These conditions select for bifidobacteria, which ferment lactose to yield acetic acid and grow optimally under the acidic conditions (pH 5–5.5) that they produce in the stool. Infants who are fed cow's milk, which has a greater buffering capacity, tend to have less acidic stools and a flora more similar to that found in the colon of the weaned infant or the adult. These findings also apply to infants fed some artificial formulas.

## Respiratory Tract

*S. aureus* is carried in anterior nares

Nasopharynx is often a site of carriage of potential pathogens

The external 1 cm of the anterior nares is lined with squamous epithelium. The nares have a flora similar to that of the skin except that it is the primary site of carriage of a pathogen, *Staphylococcus aureus*. About 25 to 30% of healthy people carry this organism as either resident or transient flora at any given time. The organism may spread to other skin sites or colonize the perineum; it can be disseminated by hand-to-nose contact, by desquamation of the epithelium, or by droplet spread during upper respiratory infection. The nasopharynx has a flora similar to that of the mouth; however, it is often the site of carriage of potentially pathogenic organisms such as pneumococci, meningococci, and *Haemophilus* species.

Lower tract is protected by mucociliary action

The respiratory tract below the level of the larynx is protected in health by the action of the epithelial cilia and by the movement of the mucociliary blanket; thus, only transient inhaled organisms are encountered in the trachea and larger bronchi. The accessory sinuses are normally sterile and are protected in a similar fashion, as is the middle ear by the epithelium of the eustachian tubes.

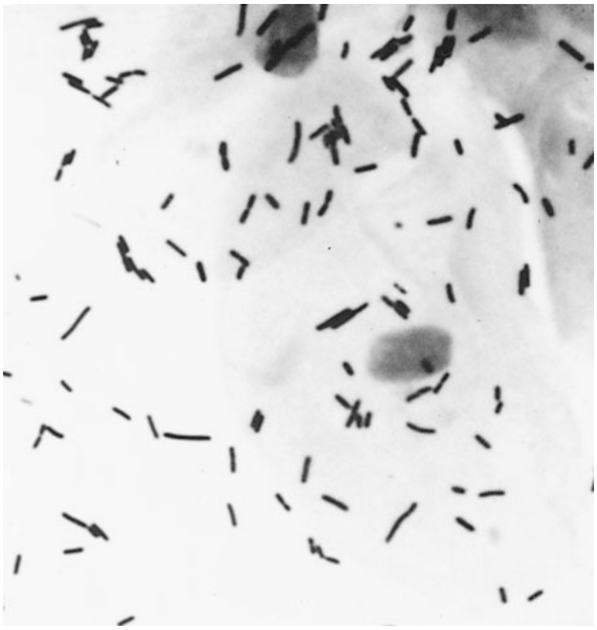

FIGURE 9–2
Smear of normal adult vagina, showing predominant large elongated lactobacilli and squamous epithelial cells.

## Genitourinary Tract

The urinary tract is sterile in health above the distal 1 cm of the urethra, which has a scanty flora derived from the perineum. Thus, in health the urine in the bladder, ureters, and renal pelvis is sterile. The vagina has a flora that varies according to hormonal influences at different ages. Before puberty and after menopause, it is mixed, nonspecific, relatively scanty, and contains organisms derived from the flora of the skin and colon. During the childbearing years, it is composed predominantly of anaerobic and microaerophilic members of the genus *Lactobacillus,* with smaller numbers of anaerobic Gram-negative rods, Gram-positive cocci, and yeasts that can survive under the acidic conditions produced by the lactobacilli. These conditions develop because glycogen is deposited in vaginal epithelial cells under the influence of estrogenic hormones and metabolized to lactic acid by lactobacilli. This process results in a vaginal pH of 4 to 5, which is optimal for growth and survival of the lactobacilli, but inhibits many other organisms. The consistency of the lactobacillary adult flora is seen in Gram-stained preparations of vaginal smears (Fig 9–2).

Bladder and upper urinary tract are sterile

Hormonal changes affect the vaginal flora

Use of epithelial glycogen by lactobacilli produces low pH

## ROLE OF THE NORMAL FLORA IN DISEASE

Many species among the normal flora are opportunists in that they can cause infection if they reach protected areas of the body in sufficient numbers or if local or general host defense mechanisms are compromised. For example, certain strains of *E. coli* can reach the urinary bladder by ascending the urethra and cause acute urinary tract infection, usually in sexually active women. Perforation of the colon from a ruptured diverticulum or a penetrating abdominal wound releases feces into the peritoneal cavity; this fecal contamination may be followed by peritonitis, caused primarily by facultative members of the flora, and by intraabdominal abscesses, caused primarily by Gram-negative anaerobes. Viridans streptococci from the oral cavity may reach the bloodstream as a result of physiologic trauma or injury (eg, tooth extraction) and colonize a previously damaged heart valve, initiating bacterial endocarditis (see Chapter 68). These and other diseases, such as actinomycosis, result from displacement of normal flora into body cavities or tissues.

Flora that reach sterile sites may cause disease

Mouth flora may reach heart valves by transient bacteremia

Reduced specific immunologic responses, defects in phagocytic activity, and weakening of epithelial barriers by vitamin deficiencies can all result in local invasion and disease by normal floral organisms. This source accounts for many infections in patients whose defenses are compromised by disease (eg, diabetes, lymphoma, and leukemia) or

Compromised defense systems increase the opportunity for invasion

Mouth flora plays a major role in dental caries

by cytotoxic chemotherapy for cancer. One specific local infection of this type is Vincent's angina of the oral mucosa, a local invasion and ulceration apparently caused by the combined action of oral spirochetes and members of the genus *Fusobacterium*. Death after lethal radiation exposure usually results from massive invasion by normal floral organisms, particularly those of the intestinal tract. Caries and periodontal disease are both caused by organisms that are members of the normal flora. They are considered in detail in Chapter 62.

Nonspecific "toxic" effects of colonic flora are postulated

Early in the 20th century, it was widely believed that the normal flora of the large intestine was responsible for many "toxic conditions," including rheumatoid arthritis, degenerative diseases, and a range of conditions now recognized as psychosomatic. Ritualistic purging and colonic lavage flourished, particularly at expensive mineral spas. At the height of this misdirected attack on the normal flora, some London patients were even subjected to colectomy as a cure for thyroid nodules. These notions persist in the form of the alleged beneficial effect of enemas and colonic lavages.

Blind-loop overgrowth may cause fat malabsorption and $B_{12}$ deficiency

Colonization of jejunum occurs in tropical sprue

However, more recently, attention has again been focused on the less specific contributions of the normal flora to health and disease. In patients with large or multiple blind-ended diverticula in the small intestine, heavy colonization by the anaerobic intestinal flora may occur. This colonization results in bacterial deconjugation of bile salts needed for absorption of fat and fat-soluble vitamins and also in competition for vitamin $B_{12}$. Similar situations sometimes occur in the elderly when the small intestine is invaded by colonic flora. If the primary cause cannot be eliminated surgically, these conditions can be ameliorated with antibiotic therapy and fat-soluble vitamin supplements. An analogous situation occurs in tropical sprue, in which secondary colonization of the jejunum by facultative Gram-negative enteric bacteria leads to fat malabsorption and vitamin $B_{12}$ and folic acid deficiencies. It has been postulated that the higher colon cancer rates in those consuming Western as opposed to Asian diets may be a result of greater production by members of the normal flora of carcinogens such as nitrosamines and bile acid derivatives.

Ammonia production and bypass lead to hepatic encephalopathy

Under certain conditions, a "toxemia" can result from the action of the normal colonic flora. In severe hepatic cirrhosis, the portal circulation may be partially diverted to the systemic circulation. The detoxification by the liver of ammonia produced by bacterial action on protein residues is bypassed, and severe dysfunctions of the central nervous system (hepatic encephalopathy) can result. This problem can be ameliorated with a strict low-protein diet.

## BENEFICIAL EFFECTS OF THE NORMAL FLORA

### Priming of Immune System

Sterile animals have little immunity to microbial infection

Low exposure correlates with asthma risk

Organisms of the normal flora play an important role in the development of immunologic competence. Animals delivered and raised under completely aseptic conditions ("sterile" or gnotobiotic animals) have a poorly developed reticuloendothelial system, low serum levels of immunoglobulins, and none of the antibodies to normal floral antigens that often cross-react with those of pathogenic organisms and confer a degree of protection against them. There is evidence of immunologic differences between children who are raised under usual conditions and those that minimize the exposure to diverse flora. Some studies have found a higher incidence of asthma in the more isolated children.

### Exclusionary Effect

Breastfeeding and a bifidobacterial flora have a protective effect

*Lactobacillus* vaginal flora can protect against fomite-transmitted gonorrhea

The normal flora produces conditions that tend to block the establishment of extraneous pathogens and their ability to infect the host. The bifidobacteria in the colon of the breast-fed infant produce an environment inimical to colonization by enteric pathogens; this protective effect is aided by ingested maternal IgA. Breastfeeding has clearly been shown to help protect the infant from enteric bacterial infection. The normal vaginal flora has a similar protective effect. Before the introduction of antibiotic therapy, researchers found that synthetic estrogen therapy controlled institutional outbreaks of fomite-transmitted gonococcal vulvovaginitis in prepubertal girls. This treatment led to glycogen deposition in the vaginal epithelium and establishment of a protective lactobacillary flora. The possible hazard of such therapy in this population was not then recognized.

Antibiotic therapy, particularly with broad-spectrum agents, may so alter the normal flora of the gastrointestinal tract that antibiotic-resistant organisms multiply in the relative ecologic vacuum, sometimes causing significant infections, particularly in immunocompromised patients. The pathogenic yeast *Candida albicans,* a minor component of the normal flora, may multiply dramatically and cause superficial fungal infections in the mouth, vagina, or anal area. Pseudomembranous colitis results from overproliferation of a toxin-producing anaerobe, *Clostridium difficile,* which has a selective advantage in the presence of antibiotic therapy. It may be resistant to several antibiotics that act on other members of the colonic flora, allowing *C. difficile* to increase from a minor to a major component. Its toxins cause diarrhea and direct damage to the colonic epithelium.

Antibiotic therapy may provide a competitive advantage for pathogens

The exclusionary effect of the flora in health has been demonstrated in numerous experiments on gnotobiotic and antibiotic-treated animals. For example, *C. albicans* attaches to oral epithelial cells of germ-free rats; however, prior colonization with certain viridans streptococci that attach to similar epithelial cells prevents establishment of *C. albicans*. In another experiment, the infecting oral dose for mice of streptomycin-resistant *Salmonella* was approximately $10^5$ organisms in untreated animals. Oral streptomycin treatment, which inhibits many members of the normal flora, reduced the infecting dose by approximately 1000-fold.

Exclusionary effect makes entrance of pathogens more difficult

## Production of Essential Nutrients

In ruminants, the action of the extensive anaerobic flora in the rumen is essential to the nutrition of the animal. The flora digests cellulose to usable form and provides many vitamins, including 70% of the animal's vitamin B requirements. In humans, members of the vitamin B group and vitamin K are produced by the normal flora; however, except for vitamin K the amounts available or absorbed are small compared with those in a well-balanced diet. Bacterial vitamin production is reduced during broad-spectrum antibiotic therapy, and supplementation with vitamin B complex is indicated in malnourished individuals.

Some vitamins are produced by members of the normal flora

# MANIPULATION OF THE NORMAL FLORA

Attempts to manipulate the normal flora have often been fruitless and have sometimes been dangerous. Exclusion of the normal flora has been effective in patients whose immunologic defenses are massively compromised (eg, following the whole-body irradiation used in bone marrow transplantation). Significant effects require the use of antimicrobics, sterilization of food and supplies, air filtration, and strict aseptic nursing procedures. These conditions substantially reduce the risk of infection during highly vulnerable periods.

Efforts to control which organisms make up the flora have been more problematic. During nursery outbreaks of *S. aureus* infections in the 1950s, deliberate colonization of an infant's nares with *S. aureus* 502A, a strain of low virulence, was attempted as a control measure. This approach was based on the hope that it would exclude more virulent strains of *S. aureus*. Unfortunately, some infections occurred with the 502A strain.

One area where there has been some success in promoting colonization with "good" flora is with lactobacilli in the intestinal tract. Elie Metchnikoff originally suggested that the longevity of Bulgarian peasants was attributable to their consumption of large amounts of yogurt; the live lactobacilli in the yogurt presumably replaced the colonic flora to the general benefit of their health. This notion persists today in the alleged benefit of natural (unpasteurized) yogurt, which contains live lactobacilli. Although we now know that lactobacillary replacement of the flora of the adult colon does not take place so easily, there have been some successes with capsules containing lyophilized bacteria. In some studies, administration of preparations containing a particular strain of *Lactobacillus* (*L. rhamnosus* strain GG, LGG) has reduced the duration of rotavirus diarrhea in children and prevented relapses of antibiotic-associated diarrhea caused by *C. difficile*. LGG suppositories have also been used to prevent recurrent vaginitis caused by the yeast *C. albicans* with mixed results. A better understanding of the relationship between virulence and the extremely complex interactions of the normal flora is needed for the rational deployment of "good" flora to our benefit.

## ADDITIONAL READING

Alvarez-Olmos MI, Oberhelman RA. Probiotic agents and infectious diseases: A modern perspective on a traditional therapy. *Clin Infect Dis* 2001;32:1567–1576. This review gives a critical analysis of the effectiveness of using “good” flora to prevent or treat disease.

Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;343:538–543. This study suggests on epidemiologic grounds that failure to prime the immune system by exposure to the flora of other individuals is associated with an increased risk for asthma.

Rosebury T. *Life on Man*. New York: Berkeley; 1970. A delightful, wry, and instructive paperback. Highly recommended for recreational reading.

CHAPTER 10

# Host-Parasite Relationships

STANLEY FALKOW

Infectious diseases have been the major causes of human death and suffering throughout history. Indeed, infectious diseases remain the leading cause of death throughout a world in which most of the population does not have the luxury of living long enough to succumb to the chronic diseases of aging. The major factors that have influenced the emergence of infectious diseases as the leading cause of morbidity and mortality historically are discussed below.

## EMERGENCE OF INFECTIOUS DISEASE

The presence of human populations is large enough to sustain and amplify parasites, thus contributing to increased disease. Humans have lived in communities large enough to perpetuate parasites only for about 10,000 years, barely a blink of the eye in the time frame of evolution. Thus, many of the human diseases that have been predominant historically probably did not exist in early humans. Many of the well-known infectious diseases of humans are very recent in the evolutionary sense. For example, the great Black Death of the 14th century, just 700 years ago, led to the death of approximately one third to one half of the known human population. The effects of plague on the human population are still largely unknown. In terms of the evolution of the human gene pool, those that died were likely as important as those that survived. It has been suggested that the resistance of some Caucasian populations to the recent scourge of human immunodeficiency virus (HIV) may actually reflect the genetic consequences of survival from some infectious disease prevalent 20 generations ago. However, some diseases such as treponematosis, mycobacterial infection, infections caused by some protozoans and worms, and diseases caused by herpesviruses, likely afflicted early humans because of their latency and their tendency to reactivate over long periods of time.

Growth and changers in human populations may influence prevalence

Poverty, with its crowding, unsanitary conditions, and often malnutrition, leads to an increased susceptibility to infection and disease. War, famine, civil unrest, and, of course, epidemic disease lead to a breakdown in public infrastructure and the increased incidence of infectious diseases.

Poverty → disease

War → disease

In the history of human civilization, one of the most important facets of the evolution of human infectious diseases was the domestication of animals, which began about 12,000 years ago. There is good cause to think many of the best-known epidemic diseases evolved from animal species and only became adapted to humans rather recently. We are still in an evolutionary dynamic with our large and small parasites; the relationship between humans and the microbes they are heir to has not stopped evolving. Perhaps it

Animal domestication is important

never will. While microbes have evolutionary flexibility, humans try to meet the onslaught of infection with genes that are essentially still those of primitive hunter-gatherers. The actual large-scale domestication of animals has slowed, and it has been replaced by the encroachment of human populations into the domain of animal, insect, and marine species all over the globe. It is little wonder that our deliberate destruction of predators and the outgrowth of human populations into previously virgin land with its attendant destruction of habitat lead to the emergence of "new" diseases such as Lyme disease; Legionnaires' disease; and likely, acquired immunodeficiency syndrome (AIDS).

## THE OUTCOME OF INFECTION

Infectious diseases are complex. They involve much more than growth of microbes or parasitic animals in the body. The factors that determine the initiation, development, and outcome of an infection involve a series of complex and shifting interactions between the invading organism and the host, which can vary with different infecting organisms. These interactions include the following:

Multiple steps influence the outcome

1. The organism's ability to breach host barriers and to evade destruction by innate local and tissue host defenses.
2. The organism's biochemical tactics to replicate, to spread, to establish infection, and to cause disease.
3. The microbe's ability to transmit to a new susceptible host.
4. The body's innate and adaptive immunologic ability to control and eliminate the invading parasite.

Broad principles apply

Despite the complexity of interactions between different parasites and hosts, several components of pathogenic processes and principles have broad application to infectious diseases and are described in this chapter. Details of individual organisms and diseases are given in subsequent chapters. Basic mechanisms of specific immune responses are discussed in Chapter 8 and are not recapitulated here. In considering this topic, it is essential to bear in mind that the ability of an organism to infect or to cause disease depends on the susceptibility of the host. There are remarkable species differences in host susceptibility to many infections. For example, dogs do not get measles, nor do humans get canine distemper, although the causative viruses are closely related.

## WHAT IS A PATHOGEN?

Humans live in a world filled with microbes

Most microbes are beneficial, not harmful

In medicine, we define a pathogen as any microorganism capable of causing disease. The emphasis is on disease, not the microorganism. However, from the microbial standpoint, being pathogenic is a strategy for survival and simply one more remarkable example of the extraordinary diversity of the microbial world. Humans, including physicians, probably spend too little time reflecting on the fact that we are home to a myriad of other living creatures. From mouth to anus, from head to toe, every millimeter of our cells that is exposed to the outside world has a rich biological diversity. From the mites that inhabit the eyebrows of many of us to the seething cauldron of over 600 species of bacteria that inhabit our large bowel, we are a veritable garden of microorganisms. Most of these microorganisms are not only innocuous but play a useful, if unseen, role. Not only do they provide us with protection against the few harmful microorganisms that we encounter each day, but they also give us some vitamins and nutrients and help digest our food. We have harbored them so long in our evolution that they are even a necessary part of the developmental pathways required for the maturation of our intestinal mucosa and our innate local immune system.

Commensals exist in mutual comfort

Most human microbes are **commensal;** that is, they eat from the same table that we do. These microbes are constant companions and often depend on humans for their existence. Although humans do not appear to be absolutely dependent on microbes for life (at least the cultivatable ones we know), we exist more comfortably with microbes than without them. We also encounter transient microbes, which are just passing through or on us, so to speak. Some commensal transient species may be **opportunistic pathogens.** These organisms can cause disease only if one or more of the usual defense mechanisms humans have evolved to restrict microorganisms from their usually sterile internal organs and tissue are breached by accident, by intent (eg, surgery), or by an underlying metabolic or an infectious disorder (eg, AIDS). Nevertheless, a small group of microorganisms often causes infection and overt disease in seemingly normal individuals. These are the **primary pathogens** such as the common cold virus, the mumps virus, the typhoid bacillus, gonococcus, the tubercle bacillus, and the treponema of syphilis. Each organism is adapted exclusively to humans; other pathogens such as *Salmonella typhimurium,* a common cause of human food poisoning, can cause disease both in humans and other animals, birds, and even reptiles.

Opportunists can cause disease under certain circumstances

Pathogens regularly cause overt disease

What is the difference between a commensal, an opportunist, and a primary pathogen? All of these organisms can cause disease under the proper circumstances. One distinction to make between an opportunistic pathogen and a primary pathogen is on the basis of the essentiality of the host for the long-term survival of a microbe. Long-term survival in a primary pathogen is absolutely dependent on its ability to replicate and to be transmitted in a particular host; however, this is not necessarily the case for a number of the opportunistic pathogens that infect humans. The major distinction that emerges is that primary pathogens have evolved the genetic ability to breach human cellular and anatomic barriers that ordinarily restrict or destroy commensal and transient microorganisms. Thus, pathogens can inherently cause damage to cells to gain access by force to a new unique niche that provides them with less competition from other microorganisms, as well as a ready new source of nutrients. For microorganisms that inhabit mammals as an essential component of their survival tactic, success can be measured by the capacity to multiply sufficiently to be maintained or be transmitted to a new susceptible host. This is true for commensal and pathogen alike. However, if the pathogen gains a new niche free of competition and rich in nutrients, it also faces a more hostile environment designed by evolution to restrict microbial entry and, indeed, to destroy any intruders that dare to enter these protected regions. Thus, pathogens have not only acquired the capacity to breach cellular barriers, they also have, by necessity, learned to circumvent, exploit, subvert, and even manipulate our normal cellular mechanisms to their own selfish need to multiply at our expense.

Pathogens must move on to another host

The strategy for survival of a pathogen requires infection (persistence, usually by multiplication on or within another living organism). Disease (ie, the overt clinical signs and symptoms of damage that occur in a host as a result of its interaction with an infectious agent) may not be an inevitable outcome of the host-parasite interaction. Rather, the requirement for a microbial infection is sufficient multiplication by the pathogen to secure its establishment within the host by transient or long-term colonization or to bring about its successful transmission to a new susceptible host. Thus, many (or most) common infections are inapparent and asymptomatic. Symptoms of disease can reflect part of the microbe's strategy for survival within the host. For example, coughing promotes the transmission of the tubercle bacillus and influenza virus, and diarrhea spreads enteric viruses, protozoa, and bacteria.

Features of disease may be linked to transmission

Physicians often use the terms virulent and pathogenic interchangeably. Originally, **virulence** was used as a comparison of pathogenicity in the quantitative sense, and this use of the term is still preferred. For example, the bacterial species *Haemophilus influenzae* is a common inhabitant of the upper respiratory tract of humans. Members of this species regularly cause middle-ear infection and sinusitis in children and bronchitis in smokers, but one variety of *H. influenzae* (those with capsule type b) can cause systemic disease (meningitis and epiglottitis). All *H. influenzae* are pathogenic, but *H. influenzae* type b is more virulent.

Virulence expresses degrees of pathogenicity

## CHANGES IN MICROBIAL PATHOGENICITY

Many of the major public health crises of the past two decades have been infectious in origin. If we examine them closely, many can be seen to be a natural consequence of human behavior and progress. For example, Legionnaires' disease can be traced to subtle differences in human behavior and social convention. *Legionella pneumophila* is widely

Human progress may enhance spread of some diseases found in nature as an infectious agent of predatory protozoa and is normally found in potable water supplies throughout the world. But showers and other widespread aerosolization technology (eg, spray devices for produce in supermarkets) can introduce the bacteria into the alveolus of the lung. *Legionella* finds a new niche in the human phagocytic macrophage instead of its usual protozoan hosts *Acanthamoeba* or *Hartmannella.* The microbe is programmed to replicate, and the consequence is characterized as a new or emerging infectious disease. Women in our society asked for more absorbent tampons to achieve more social freedom and unwittingly, American commerce supplied a product that helped select for certain strains of staphylococcus. Another new emerging disease, toxic shock syndrome (TSS) was recognized and caused near panic.

Aerosols spread *Legionella*

TSS is linked to tampons

These examples are not meant to turn our attention away from the pathogenic traits of the disease-causing microbes, but it seems true, on reflection, that humans, with their technology and social behavior, have played a significant role in providing pathogenic microbes with new venues for their wares. Food poisoning by *Escherichia coli* O157:H7, *Campylobacter,* and *Salmonella* arise as much from food technology and modern food distribution networks than from any fundamental change in the virulence properties of the bacteria in question. HIV, Hantavirus, and Lyme disease seem likely to be a consequence of the encroachment of humans on previously undisturbed ecological niches and the increased likelihood of human contact with animal species and their carried microorganisms. In the case of HIV, the expansion of rapid travel throughout the globe magnified this consequence. No part of our planet is more than 3 days away by air travel, a fact known and feared by all public health officials.

*E. coli* O157:H7 is spread by food processing

Today, physicians deal more and more with opportunists because our population is getting older, and the practice of medicine keeps individuals alive longer by surgical procedures and powerful drugs that affect the immune status. As a consequence, in the Western world, microorganisms that a scant 40 years ago were considered harmless commensals or environmental isolates are now feared opportunistic pathogens. Many of the primary pathogens such as measles virus are controlled now by immunization. One view is that infectious diseases are under control. Another view is that the host–parasite relationship is still in a dynamic state. Just as many people die of infection as did 40 years ago; they just die later and because of different infectious agents. It is important to understand that for most of the world, the "classic" pathogens of history such as malaria, the tubercle and leprosy bacillus, and the cholera vibrio, together with newcomers such as HIV, are the leading causes of human misery and death.

Opportunists attack vulnerable populations

Classic pathogens are dominant in most of the world

## TOWARD A GENETIC AND MOLECULAR DEFINITION OF PATHOGENICITY

The classic investigation of pathogenicity has been based on linking natural disease in humans with experimental infection produced by the same organism. The analysis of bacterial virulence determinants usually was the result of the comparative analysis of different clinical isolates of the same species that were either virulent or avirulent in a particular model system. This led to speculation about the potential role of a number of microbial traits as virulence determinants.

Comparison of strains of varying virulence is classic approach

This comparative approach now has given way to mutational analysis within a single or limited number of strains of a pathogenic species. The goal is to obtain a single, defined genetic change that alters a single virulence property and affects the pathogenesis of infection or the ability of the organism to cause pathology in an appropriate model system. The advances in microbial genetics, DNA biochemistry, and molecular biology have made it possible to apply a kind of molecular Koch's postulates to the analysis of virulence traits.

**1.** The phenotype or property under investigation should be associated significantly more often with pathogenic strains of a species than with nonpathogenic strains.
**2.** Specific inactivation of the gene or genes of interest associated with the suspected virulence trait should lead to a measurable decrease in virulence.
**3.** Restoration of pathogenicity or full virulence should accompany replacement of the mutated allele with the original wild-type gene.

Genetic manipulation can inactivate and restore virulence

This simplistic goal is not always possible because it is dependent on a suitable infection model in which to test a microorganism. The ideal model can be infected by a natural route using numbers analogous to those seen in human infection and can duplicate the relevant pathology observed in the natural host. Except for other primates, such models do not exist for pathogens that are restricted to humans. For example, it is still difficult to assess the role of IgA1 protease in the pathogenicity of *Neisseria gonorrhoeae,* because the enzyme works only on human IgA1 and the microorganism is an exclusive human pathogen.

Strict human pathogens are more difficult to study

Despite these technical limitations, there has been a revolution over the past decade in understanding of the basic pathogenic mechanisms and how microbes bring about infection and disease. The use of transgenic animals, reconstituted human immune systems in rodents, and the extension of cell and organ culture methods to the study of infectious agents will lead to greater understanding of the pathogenesis of infectious diseases. In parallel, new methods to visualize living microbes in tissue and to monitor genetic activity through "reporter molecules" will permit the monitoring of microbes in infected tissue in real time. The full genomic sequence of most pathogenic microbial species will be completed within the coming decade. This information, coupled with contemporary technology of DNA arrays and the parallel knowledge about the human genome, soon will allow examination of the expression of every bacterial gene and a representative expression of host genes in both experimental infection models and in samples obtained from infected patients. This knowledge will continue to impact how infectious diseases are diagnosed, treated and prevented in the not-too-distant future.

Transgenic animals allow monitoring of virulence determinants

## ATTRIBUTES OF MICROBIAL PATHOGENICITY

Whether a microbe is a primary or opportunistic pathogen, it must be able to enter a host; find a unique niche; avoid, circumvent or subvert normal host defenses; and multiply. To be successful, a primary pathogen also must be transmitted to a new susceptible host or establish themselves in the host for an extended period of time and eventually be transmitted.

### Entry

Like all other living organisms, humans must maintain contact with the environment to see, breathe, ingest food, reproduce, and eliminate wastes. Consequently, each of the portals in the body that communicates with the outside world becomes a potential site of microbial entry. Human and other animal hosts have various protective mechanisms to prevent microbial entry (Table 10–1). A simple, although relatively efficient, mechanical barrier to microbial invasion is provided by intact epithelium, the most effective of which is the stratified squamous epithelium of the skin with its superficial cornified anucleate layers. Organisms can gain access to the underlying tissues only by breaks or by way of hair follicles, sebaceous glands, and sweat glands that traverse the stratified layers. The surface of the skin continuously desquamates and thus tends to shed contaminating organisms. The skin also inhibits the growth of most extraneous microorganisms because of low moisture, low pH, and the presence of substances with antibacterial activity.

Microbes gain access from the environment

Skin is a major protective barrier

A viscous mucus covering protects the epithelium lining the respiratory tract, the gastrointestinal tract, and urogenital system secreted by goblet cells. Microorganisms become trapped in the mucus layer and may be swept away before they reach the epithelial cell surface. Secretory IgA (sIgA) secreted into the mucus and other secreted antimicrobials such as lysozyme and lactoferrin aid this cleansing process. Ciliated epithelial cells constantly move the mucus away from the lower respiratory tract. In the respiratory tract, particles larger than 5 $\mu$m are trapped in this fashion. The epithelium of the intestinal tract below the esophagus is a less efficient mechanical barrier than the skin, but there are other effective defense mechanisms. The high level of hydrochloric acid and gastric enzymes in the normal stomach kill many ingested bacteria. Others are susceptible to pancreatic digestive enzymes or to the detergent effect of bile salts. Similarly, the multilayered transitional epithelium of the urinary tract uses the flushing effect of urine and its relatively low pH as additional defense mechanisms to limit microbial entry and growth.

Secretions coat mucosal epithelium

Acids and enzymes aid in cleansing

TABLE 10–1

**Nonspecific Defenses Against Colonization with Pathogens**

| Site | Mechanical Barrier | Ciliated Epithelium | Competition by Normal Flora | Mucus | sIgA | Lymphoid Follicles | Low pH | Flushing Effects of Contents | Peristalsis | Special Factors |
|---|---|---|---|---|---|---|---|---|---|---|
| Skin | +++ | − | + | − | − | − | ++ | − | − | Fatty acids from action of normal flora on sebum |
| Conjunctiva | ++ | − | − | − | + | − | − | +++ | − | Lysozyme |
| Oropharynx | +++ | − | +++ | − | + | Yes | − | ++ | − | |
| Upper respiratory tract | ++ | + | +++ | ++ | ++ | Yes | − | + | − | Turbinate baffles |
| Middle ear and paranasal sinuses[a] | ++ | +++ | − | ++ | ? | − | − | + | − | |
| Lower respiratory tract[a] | ++ | +++ | − | ++ | ++ | Yes | − | − | − | Mucociliary escalator, alveolar macrophages; cough reflex |
| Stomach | ++ | − | − | ++ | − | − | +++ | + | + | Production of hydrochloric acid |
| Intestinal tract | ++ | − | +++ | +++ | +++ | Yes | − | + | +++ | Bile; digestive enzymes |
| Vagina | +++ | − | +++ | + | + | − | +++ | − | − | Lactobacillary flora ferments epithelial glycogen |
| Urinary tract[a] | ++ | − | − | − | + | − | + | +++ | − | |

*Abbreviations:* +, ++, +++ = relative importance in defense at each site; − = unimportant.

[a] Sterile in health.

Urinary tract infections are much more common in women than men because the short urethra in females allows easier passage of organisms to the bladder; such infections in women are often associated with sexual intercourse.

Pathogenic organisms have evolved mechanisms to capitalize on each of these human sites of environmental contact as points of entry. Removal of the epithelial barrier and normal host cell functions makes human into the victims of opportunists. One natural method of bypassing the skin is direct inoculation by insect bites; several organisms use this route, including the plague bacillus *Yersinia pestis* and the malarial parasite. These microorganisms, which must spend part of their lives in remarkably different environments than their mammalian hosts, have adapted mechanisms for survival. Another means in the modern world of bypassing the skin is through the deliberate inoculation used by drug addicts who suffer from a particular constellation of infectious disease agents as a result.

Pathogens are adapted to mucosal environments

Insect injection allows pathogens to bypass barriers

We still know very little about the microbial factors essential to ensure infectious transmission from host to host. Obviously, microbes adapted to life in humans have evolved to take advantage of existing avenues of contact between their hosts. Dissemination by aerosol is common, but success is more than just random chance; the parasite must design itself for the rigors of atmospheric drying and other environmental factors. The virus of the common cold must exist on inanimate objects (fomites) waiting for a hand to touch and carry them to the conjunctiva or nasopharynx. The burden on an enteric pathogen that follows the fecal–oral route is substantial: feces, mouth to stomach to small bowel to large bowel and back to the cold cruel world in stool. Thus, bacteria causing enteric infection are exposed to extremes of temperature, pH, bile salts, digestive enzymes, and a myriad of competing microorganisms. Sexually transmitted pathogens ordinarily are delivered by direct inoculation onto mucosal surfaces. This microbial strategy avoids life in the external environment but is not without its own set of special requirements to overcome changing pH, mucus obstruction, anatomic barriers, local antibodies, and phagocytic cells.

Environmental survival facilitates host-to-host transmission

Sexual transmission is direct

All of the factors in the initial encounter of the host with the parasite can be assessed to some degree by measuring the infectious dose of the organism. How many organisms must be given a host to ensure infection in some proportion of the individuals? The measure of the infectious dose-50 ($ID_{50}$) for several pathogens is shown in Table 10–2. It is a simple measurement of a very complex interaction. Moreover, it is somewhat misleading, as the endpoint is disease in human volunteers or death (a rigorous endpoint) in animal experiments.

Infection is dose-related

## Adherence: The Search for a Unique Niche

The first major interaction between a pathogenic microorganism and its host entails attachment to an eukaryotic cell surface. In its simplest form, adherence requires the participation of two factors: a receptor on the host cell and an **adhesin** on the invading microbe. Most viruses attach specifically to sites on target cells through an envelope protein. For example, the influenza viruses attach specifically to neuraminic acid–containing

Molecular adhesins attach to surface receptors on host cells

TABLE 10–2

### Dose of Microorganisms Required to Produce Infection in Human Volunteers

| Microbe | Route | Disease-Producing Dose |
|---|---|---|
| Rhinovirus | Pharynx | 200 |
| *Salmonella typhi* | Oral | $10^5$ |
| *Shigella* spp. | Oral | 10–1000 |
| *Vibrio cholerae* | Oral | $10^8$ |
| *V. cholerae* | Oral + $HCO_3^-$ | $10^4$ |
| *Mycobacterium tuberculosis* | Inhalation | 1–10 |

glycoprotein receptors on the surface of respiratory cells before penetrating to the interior of the cell. Bacteria, like viruses, generally have protein structures on their surface that recognize either a protein or a carbohydrate moiety on the host cell surface. Finding the correct host cell surface in many cases appears to be a probability event related to the infectious dose. Because the mucosal surface is constantly bathed by a moving fluid layer, it is not surprising that many bacteria that infect the bladder or gastrointestinal tract are motile, and some (such as the typhoid bacillus) may use chemotaxis (see Chapter 3) to home in on the correct host cell surface. Some bacteria use mucolytic enzymes to reach epithelial surfaces.

Some bacterial adherence may involve hydrophobic interruptions between nonpolar groups present on the microbe and host cell. Alternatively, one can envision cationic bridging between cells. Such interactions lack the specificity seen in most host–pathogen interactions. Rather, pathogens most often employ highly specific receptor-ligand binding. In the last decade, it has become clear that most pathogenic microorganisms have more than a single mechanism of host cell attachment, which is not just a redundant feature. More often it reflects that pathogenic microbes require different types of adherence factors depending on their location and the types of host cells they may encounter. Thus, bacteria may employ one set of adhesins at the epithelial surface but respond with a different set when they encounter cells of the immune system. Finally, not all adhesins are essential virulence factors; they may play a role in survival outside of a host or add to the biology of the microbe outside of its pathogenic lifestyle.

Many pathogens have multiple attachment mechanisms

Bacterial adhesins can be divided into two major groups: pili (fimbriae) and nonpilus adhesins (afimbrial adhesins). The pili of many Gram-negative bacteria bind directly to sugar residues that are part of glycolipids or glycoproteins on host cells or act as a protein scaffold to which another more specific adhesive protein is affixed. One of the major features among diverse pili is conservation of the molecular machinery needed for pilus biogenesis and assembly onto the bacterial surface. One of the best-studied examples of pilus assembly is P-pili (pyelonephritis-associated pili), which are encoded by *pap* genes. *E. coli* strains that express P-pili are associated with pyelonephritis, which arises from urinary tract colonization and subsequent infection of the kidney. It is thought that P-pili are essential adhesins in this disease process. The *pap* operon is a useful paradigm, because it contains many conserved features found among various pilus operons. Two molecules guide newly synthesized pilus components to the bacterial surface. The major subunit of the pilus rod is PapA, which is anchored in the bacterial outer membrane by PapH. At the distal end of the pilus rod is the tip fibrillum, composed of PapE, and the actual tip adhesin, PapG, which mediates attachment to the host cell surface. Two other proteins, PapF and PapK, are involved in tip fibrillum synthesis. Although the host receptor varies for different bacterial pili, the general concepts provided by studying the P pilus operon are conserved in many other pilus systems, and components are often interchangeable. Homologous sequences to *pap* genes also have been found in genes involved in bacterial capsule and lipopolysaccharide biosynthesis.

Pili are major bacterial adhesins

P-pili are specific for urinary epithelium

Components are regulated by an operon

Although many pili look alike morphologically, there are at least five general classes in various Gram-negative bacteria that recognize different entities on the host cell surface. Thus, although *pap*-like sequences are common throughout Gram-negative adhesins, other families of pili use alternative biogenesis and assembly machinery to form a pilus. One such group, type IV pili, is found in diverse Gram-negative organisms, including the causal agents of gonorrhea and cholera. Type IV pili subunits contain specific features, including a conserved, unusual amino-terminal sequence that lacks a classic leader sequence and, instead, generally utilizes a specific leader peptidase that removes a short, basic peptide sequence. Several possess methylated amino termini on their pilin molecules and usually contain pairs of cysteines that are involved in intrachain, disulfide bond formation near their carboxyl termini; however, analogous to the P-pilus tip adhesin, a separate tip protein may function as a tip adhesin for type IV pili. The host receptor that a pathogenicity-associated adhesin recognizes probably determines the tissue specificity for that adhesin and bacterial colonization or persistence; of course, other factors also may make a contribution. The location of the adhesin at the distal tip of pili ensures adhesin exposure to potential host receptors. Alterations in the pilus subunit can also affect

Gram-negative bacteria have five types of pili

Receptor determines tissue specificity

adherence levels, and antigenic variation in the actual structural pilin protein can be an important source of antigenic diversity for the pathogen.

The pilus model of attachment is the best-known means of bacterial attachment to a host cell surface; however, nonpilin adhesins have been demonstrated in a number of bacterial species. These are often specific outer membrane proteins that form an intimate contact between the bacterial surface and the surface of the host cell. Several of these are intriguing because they resemble or "mimic" eukaryotic sequences that mediate cell–cell adhesion and adherence to the extracellular matrix. Similar classes of molecules thought to mediate adherence in the Gram-positive bacteria are surface fibrils composed of proteins and lipoteichoic acid. For example, streptococci causing pharyngitis, express an M protein–lipoteichoic acid structure believed to mediate attachment to the prevalent host cell protein, fibronectin.

Outer membrane proteins may be adhesins

Bacterial capsular polysaccharide also may mediate adherence to host cells or play an important role in binding layers of bacteria to others immediately adherent to the epithelial surface. These bacterial biofilms not only can coat the mucosal surface but play an important role in the bacterial colonization of the inert materials used as catheters.

Polysaccharide capsules and biofilms stick to surfaces

Some organisms excrete an enzyme IgA protease, which cleaves human IgA1 in the hinge region to release the Fc portion from the Fab fragment. This enzyme might play an important role in establishing microbial species at the mucosal surface, as bacteria that cleave IgA can bind the antigen-binding domain of the immunoglobulin. This is one of several cases of molecular mimicry where bacteria (and probably viruses as well) can coat themselves with a secreted host cell product. This provides a microorganism with two advantages. First, microbes use these secreted products as a bridge to adhere to cell receptors that ordinarily bind these secreted products. Second, by binding a host cell product on its surface, the microbe disguises itself from the host cell immune system.

Proteases cleave IgA

Unlike bacteria, viruses generally only have one major adhesin that they use to attach to the host cell surface and to gain entry into the cytoplasm. Otherwise, both bacteria and viruses share the same strategy: a protein structure that recognizes a specific receptor. Host cell receptors do not exist for the sole use of infectious agents; they generally are associated with important cellular functions. The adhesive molecule on the microorganism has been selected to take advantage of the host cell's biological function(s). In this way, the adhesin provides the microbe with a unique niche where the infectious agent has the greatest chance to achieve success. Presumably, a pathogen's success can be measured by the extent of multiplication subsequent to entry. Adherence is important not only during the initial encounter between the pathogen and its host but also throughout the infection cycle.

Viral adhesins exploit host cell functions

## Strategy for Survival: Avoid, Circumvent, Subvert, or Manipulate Normal Host Cell Defenses

Once a pathogenic species reaches its unique niche, it may face formidable host defense mechanisms including dangerous phagocytic cells. Such a site may be devoid of a normal heavy commensal bacterial burden precisely because it contains added defense measures not found at the usual mucosal sites. The ways by which microbes avoid, circumvent, or even subvert or manipulate such host barriers are relatively unique for each species, although certain common pathogenic tactics have begun to be appreciated. We now know that bacterial pathogenicity is a multifaceted process that can be likened to a symphony in which each part contributes to a common theme. Yet, even though pathogenic species sometimes use genetic homologs and exhibit similar tactics to outwit host defenses, each pathogen has evolved a unique style of survival—a pathogenic signature.

### *Getting into Cells*

Many pathogenic bacteria are content to fight their way to the mucosal surface, adhere, nullify local host defense factors, and multiply. However, adherence to a cellular surface may only be the first step in other infections. Some pathogenic microbes are capable of entering into and surviving within eukaryotic cells. Some organisms direct their uptake

Some pathogens enter mucosal cells or phagocytes into host cells that are not normally phagocytic, including epithelial cells lining mucosal surfaces and endothelial cells lining blood vessels. Invasion may provide a means for a microorganism to breach host epithelial barriers. Presumably, this invasion tactic ensures a protected cellular niche for the microbe to replicate or persist. Alternatively, phagocytic cells, such as macrophages, may internalize organisms actively by several mechanisms. Pathogens that survive and replicate within phagocytic cells possess additional mechanisms that enhance their survival. Even quite different organisms can employ mechanistically similar invasion strategies.

Intracellular growth and replication is an essential step for all viruses. Bacterial entry into host cells is usually divided into two broad groups. Bacteria that, like viruses, are obligate intracellular pathogens, include the typhus group *(Rickettsia)* and the trachoma group *(Chlamydia).* Other microbes such as the typhoid–paratyphoid group *(Salmonella),* the dysentery group *(Shigella),* the Legionnaires' disease bacillus *(Legionella),* and the tubercle bacillus *(Mycobacterium)* are classified as facultative intracellular pathogens and can grow as free-living cells in the environment as well as within host cells. Whereas some pathogens do whatever they can to avoid phagocytosis, these virulent facultative intracellular organisms establish themselves and replicate within the intracellular environment of phagocytes. All of these bacteria are taken up by host cells through a specific receptor-mediated, often bacterial-directed, phagocytic event. The entering bacteria initially are seen within a membrane-bound, host-vesicular structure. Yet, both the facultative and obligate bacterial pathogens can be further classified with respect to the mechanism by which they replicate intracellularly. Thus, *Shigella* and some *Rickettsia* lyse the phagosome and multiply in the nutrient-rich safe haven of the host cell cytosol. In contrast, *Salmonella, Chlamydia, Legionella,* and *Mycobacterium* remain enclosed in a host cell–derived membrane for their entire intracellular life and modulate their environment to suit their own purposes. They survive and replicate intracellularly within a host cell vacuole by thwarting the normal host cell trafficking pattern to avoid becoming fused to the hydrolytically active components of lysosomes.

Viruses and some bacteria must replicate in cells

Some replicate in cytosol, and others in host cell membranes

Generally, invasive organisms adhere to host cells by one or more adhesins but employ a class of molecules, called **invasins,** that either direct bacterial entry into cells or provide an intimate direct contact between the bacterial surface and the host cell plasma membrane. In both cases, invasins are the first step in mediating direct interaction between one or more bacterial products and host cell molecules. Invasins are adhesins in their own right, but obviously not all adhesins (such as the pili mentioned earlier) mediate entry into host cells. Invasins usually trigger or activate signals in the host cell that directly or indirectly mediate and facilitate specific membrane–membrane interaction and, in some cases, bacterial entry. For example, enteropathogenic *E. coli* and *Helicobacter pylori,* the causative agent of peptic ulcer, use contact-dependent secretory systems to actually insert bacterial proteins into the host cell membrane. This is the first step in a cascade of events that triggers a massive redeployment of host cell cytoskeletal elements. The bacteria in question do not enter the host cell but remain tightly affixed to the host cell. The molecular manipulation by the bacteria leads to a microenvironment that is essential for bacterial persistence and proliferation. The host suffers from diarrhea in one case or an inflamed gastric mucosa in the other, an unfortunate consequence for many infected hosts. Likewise, some other bacteria do not enter host cells. The typhoid bacillus and the etiologic agent of dysentery adhere intimately to the host cell surface, and, in a contact-dependent manner, directly "inject" bacterial proteins into the host cell cytoplasm, which induces a cataclysmic rearrangement of host cell actin that envelops the bacteria by a process that resembles normal macropinocytosis. Thus, ultimately, host cell cytoskeletal components and normal cellular mechanisms are exploited by bacteria to their own end. The specific tactics used by different microbes are discussed in subsequent chapters.

Invasin proteins direct entry

Proteins are inserted by contact secretion

Cell signal systems are triggered

Following cell entry, the invading bacterium immediately is localized within a membrane-bound vacuole inside the host cell. As noted, the invading pathogen organism may or may not escape this vacuole, depending on the pathogen and its strategy for survival. A small number of bacterial species appear to forcibly enter directly into host cells by a local enzymatic digestion of the host cell membrane following adherence to the cell

Phospholipase digestion facilitates cytosol entry surface. One such pathogen, *Rickettsia prowazekii,* produces phospholipases that appear to degrade the host wall localized beneath the adherent organisms, thereby enabling the pathogen to enter directly into the cytoplasm. How the bacterium controls the enzymatic degradation to prevent host cell lysis and how the host cell reseals its membrane after invasion remain uncharacterized.

Invasin binding sites can be members of the integrin family, a family of integral membrane glycoproteins mediating cell–cell and cell–extracellular matrix interactions. Integrins include the receptors for fibronectin, collagen, laminin, vitronectin, and the complement binding receptor of phagocytes. Integrins are linked to the actin microfilament system through a variety of molecules, including talin, vinculin, and $\alpha$-actinin. Thus, the binding of a microbe to an integrin or integrin-like molecule on the host surface may trigger a host cell signal that causes actin filaments to link to the membrane-bound receptor, which then generates the force necessary for parasite uptake. Understanding the cell biology of microbial invasion is still in its early stages, but once again it is important to emphasize that pathogenic microbes most often gain entry into the host cell by altering or exploiting normal host cell mechanisms.

Integrins can be receptors and links to cytoskeleton

Some viruses are internalized in much the same way. For example, rhinoviruses of the common cold use membrane-bound glycoprotein intercellular adhesion molecule 1 (ICAM-1) as a receptor. ICAM-1 is also a ligand of certain integrins. More often, as already discussed, virus particles are taken up by the receptor-mediated endocytosis mechanism (see Chapter 6), which is normally responsible for internalizing hormones, growth factors, and some important nutrients.

### *Avoiding Intracellular Pitfalls*

Intracellular pathogens enjoy a number of advantages. Besides avoiding the host immune system, intracellular localization places pathogens in an environment potentially rich in nutrients and devoid of competing microorganisms. Intracellular life is not free of difficulty. Viruses that enter by fusion are "dumped" directly into the cytoplasm where they may begin the replicative cycle. Bacteria or viruses internalized through the reorganization of the cytoskeleton find themselves in a membrane-bound vesicle in an acidic environment and may be destined for fusion with potentially degradative lysosomes. Some viruses respond to the acidic environment by changing conformation, binding to the endosomal membrane, and releasing their nucleic acid into the cytoplasm. Bacteria such as *Shigella,* the cause of bacillary dysentery, and *Listeria monocytogenes,* a causative agent of meningitis and sepsis in the very young or very old, elaborate an enzyme that dissolves away the surrounding membrane and permits the bacterium to replicate within the relative safety of the cytoplasm. Other organisms, such as the typhoid bacillus and the tubercle bacillus, apparently tolerate the initial endosome–lysosome fusion event; however, most recent evidence suggests that they then modify this intracellular compartment into a privileged niche in which they can replicate optimally. *Mycobacterium* somehow inhibit the acidification of the phagosome. Still other organisms, for example, the protozoan *Toxoplasma gondii,* inhibit the acidification of the endosomal vesicle and this, in turn, inhibits lysosomal fusion. The common theme again is that the microorganism has found a way to circumvent or to exploit host cell factors to suit its own purpose.

Intracellular site is free of competition, immune system

Resistance to degradative enzymes is needed

## Establishment: Overcoming the Host's Immune System

Once a microorganism has breached the surface epithelial barrier, it is subject to a series of nonspecific and specific processes designed to remove, inhibit, or destroy it. These defenses are complex, dynamic, and interactive. Microorganisms that reach the subepithelial tissues are immediately exposed to the intercellular tissue fluids, which have defined properties that inhibit multiplication of many bacteria. For example, most tissues contain lysozyme in sufficient concentrations to disrupt the cell wall of some Gram-positive bacteria. Other less well-defined inhibitors from leukocytes and platelets have also been described. Tissue fluid itself is a suboptimal growth medium for most bacteria and deficient in free iron. Iron is essential for bacterial growth, but it is sequestered by the body's iron-binding proteins such as transferrin and lactoferrin and is inaccessible to organisms that do not themselves produce siderophores (see Chapter 3). Virtually all pathogenic species come equipped with a means to extract the essential iron they need from the host's iron-sequestering defenses.

Subepithelial environment is different

Iron sources are important for the pathogen

Apoptosis may be induced

If an organism proceeds beyond the initial physical and biochemical barriers, it may meet strategically placed phagocytic cells of the monocyte/macrophage lineage whose function is to engulf, internalize, and destroy large particulate matter, including infectious agents. Examples of such resident phagocytic cells include the alveolar macrophages, liver Kupffer cells, brain microglial cells, lymph node and splenic macrophages, kidney mesangial cells, and synovial A cells. As noted above, many pathogens are facultative intracellular parasites that actually seek out, enter, and replicate within these phagocytic defenders. One of the most common tactics of these pathogens is to induce programmed cell death (apoptosis). This clever microbial tactic not only inactivates the killing potential of the phagocyte but also reduces the number of defenders available to inhibit other bacterial invaders. The invading bacteria that induce apoptosis obtain the added benefit that death by apoptosis nullifies the normal cellular signaling processes of cytokine and chemokine signaling of necrotic death. Hence, the myriads of microbes that infect humans and make up their normal flora are held at bay by our innate and adaptive immune mechanisms. Pathogenic bacteria, almost by definition, can overcome these biochemical and cellular shields after they breach the mucosal barrier.

Not all pathogens can deactivate the host's early warning system, inflammation. Inflammation is a normal host response to a traumatic or infectious injury. When many microorganisms multiply in the tissues, the usual result is an inflammatory response, which has several immediate defensive effects. It increases tissue fluid flow from the bloodstream to the lymphatic circulation and brings phagocytes, complement, and any existing antibody to the site of infection. Macrophage-derived interleukin-1 (IL-1) and tumor necrosis factor (TNF) stimulates or enhances these processes. The increased lymphatic drainage serves to bring microbes or their antigens into contact with the cells in the local lymph nodes that mediate the development of specific immune responses. Microorganisms that escape from a local lesion into the lymphatic circulation or bloodstream are rapidly cleared by reticuloendothelial cells or arrested in the small pulmonary capillaries and then ingested by phagocytic cells. This process is so efficient that when a million organisms are injected into a vein of a rabbit, few, if any, are recoverable in cultures of blood taken 15 minutes after injection, although the ultimate result of such clearance may not be a cure. The end results are the classic inflammatory manifestations of **swelling** (tumor), vasodilatation of surface vessels with **erythema** (rubor), **heat** (calor) from increased skin temperature, **pain** (dolor) from increased pressure and tissue damage, and **loss of function** because of reflex nerve inhibition or the pain caused by movement.

Cytokines induce inflammation

Tissues may be injured by PMNs

Function of fever is unknown

**Fever,** a frequent concomitant of inflammation, is mediated primarily by IL-1 and TNF released by macrophages. The value of fever is not completely clear; however, it increases the effectiveness of several processes involved in phagocytosis and microbial killing and frequently reduces the multiplication or replication rate of bacteria or viruses. Taken together, these host cell factors serve to produce an environment that is highly hostile to most organisms and also is hostile to adjacent normal tissue. Lysosomal enzymes, including collagenase and elastase, when released from polymorphonuclear neutrophils (PMNs) damage tissues and contribute to the enhancement of the inflammatory process; however, failure of the phagocytes to clear bacteria results in continued release of toxic products from the inflammatory exudate, which can be as damaging to the host as released bacterial virulence products. Ultimately, phagocytes kill almost all bacteria. When the invading microorganisms (or their surviving antigenic material) cannot be degraded or are resistant to removal or degradation, T cells accumulate and release lymphokines. This leads to the aggregation and proliferation of macrophages and the characteristic appearance of a nodular mass called a **granuloma,** which consists of multinucleate giant cells, epithelioid cells, and activated macrophages. Granulomas are characteristic of infections caused by the tubercle bacillus *Mycobacterium tuberculosis* and other facultative intracellular parasites.

## VIRULENCE FACTORS: TOXINS

The successful pathogen must survive and multiply in the face of these formidable host defenses. Microbial virulence factors that permit the establishment of the pathogen in the hostile host environment are essential. If these factors are lost, the capacity to infect the host or become transmitted successfully goes with them. Not surprisingly, these virulence factors are also the target(s) in the design of vaccines. The following sections provide a general overview of the classes of bacterial virulence factors that permit them to overcome host defenses. No pathogen possesses all of the classes of virulence factors, nor are all virulence factors absolutely essential for a pathogen to reach its goal of sufficient multiplication to establish itself in the host or to be transmitted to a new susceptible host.

### Exotoxins

A number of microorganisms synthesize protein molecules that are toxic to their hosts and are secreted into their environment or are found associated with the microbial surface. These exotoxins usually possess some degree of host cell specificity, which is dictated by the nature of the binding of one or more toxin components to a specific host cell receptor. The distribution of host cell receptors often dictates the degree and the breadth of the toxicity. Bacterial exotoxins, whether synthesized by Gram-positive or Gram-negative bacteria, fall into two broad classes, each of which represents a general pathogenic theme common to many bacterial species.

Secreted into their environment

#### *A–B Exotoxins*

The best known pathogenic exotoxin theme is represented by the A–B exotoxins. These toxins are divisible into two general domains. One, the B subunit, is associated with the binding specificity of the molecule to the host cell. Generally speaking, the B region binds to a specific host cell surface glycoprotein or glycolipid. The other, subunit A, is the catalytic domain, which enzymatically attacks a susceptible host function or structure. The actual biochemical structure of exotoxins varies. In some cases (diphtheria toxin), the single B subunit of the toxin is linked through a disulfide bond to the A subunit. In other cases (pertussis toxin), multiple B subunits may join with a single A enzymatic subunit. In any event, following attachment of the B domain to the host cell surface, the A domain is transported by direct fusion or by endocytosis into the host cell. Many of the most potent A–B bacterial toxins are ADP-ribosylating enzymes. Some of these affect the protein-synthesizing apparatus of the cell (diphtheria toxin, *Pseudomonas* exotoxin); others affect the cytoskeleton (*Clostridium botulinum* toxin C2) or the normal signal transduction activities of the host (*Bordetella pertussis* and *Vibrio cholerae*). It is notable that the major natural substrates of the toxin ADP-ribosyltransferases are guanine nucleotide-binding proteins (G proteins), which are involved in signal transduction in eukaryotic cells. In a very simplistic way, one can think that the ADP-ribosylating toxins are all geared to interrupt the biochemical lines of communication within and between host cells. An understanding of bacterial toxins, therefore, sheds as much light on the intimate details of normal animal cell regulation as it does on bacterial pathogenicity.

B unit binds to cell receptor

A is enzymatically active

Several bacterial toxins have been examined in exquisite detail at the biochemical level. The crystal structures of several have been "solved." In many cases, the precise amino acids making up the catalytic site of the toxin are so well known that a single amino acid substitution can be made that is sufficient to detoxify the molecule. These **toxoids** are the basis for new generations of vaccines. Given this level of biochemical sophistication, it is somewhat disconcerting to realize that the actual role of bacterial toxins in microbial pathogenicity has not been clarified. A number of the most fearsome human diseases are the result of intoxication by secreted bacterial toxins. Human disease as a consequence of an accidental contamination of a wound with the tetanus bacillus or the accidental ingestion of food contaminated with botulinum toxin is an individual human disaster, but it does not necessarily reveal the actual role of the toxin in the biology of *Clostridium tetani* or *Clostridium botulinum.* These organisms are not primary pathogens of humans, although their toxins presumably have evolved to play some role in their

Single amino acid substitutions may render inactive

Role of the toxin for the organism is unknown interaction with other eukaryotic life forms. Nontoxigenic variants of tetanus or the botulinum bacterium are totally avirulent for humans.

Some toxigenic microbes are highly adapted to humans including *Corynebacterium diphtheriae* (diphtheria), and *B. pertussis* (whooping cough). Others such as *V. cholerae* are very toxic to humans but have a reservoir, presumably on or in a marine animal. For these A–B toxins, we understand the biochemical basis for toxigenicity and the indispensability of the toxins for the pathogenicity of the microorganism. We even understand that if we immunize individuals against these toxins, we can prevent disease. What we do not fully understand is the role of the toxin in the biology of the microorganism. The toxin cannot be so potent that it rapidly kills all of the hosts that are infected. Toxins may represent the principal determinant of bacterial virulence in some species but may not be the principal determinant of infectivity; however, it seems likely that toxins play a role in the establishment of the organism in the early phases of infection or they are elaborated only if the organism "senses" danger. Thus, *V. cholerae* devoid of cholera toxin does not colonize susceptible animals as well as toxigenic organisms, nor is it as efficiently transmitted. It is possible that the effects of cholera toxin, the induced net secretion of water and electrolytes into the lumen of the bowel, make conditions right for cholera replication. On the other hand, nontoxigenic *C. diphtheriae* and *B. pertussis* can still colonize humans and be transmitted, although not as well as their toxigenic parents.

Immunization against toxin can prevent disease

Currently, molecular cloning techniques, coupled with appropriate infection models, are leading to the elucidation of the roles of some toxins in the pathogenesis of infections. Not all toxins are essential for pathogenicity. For example, *Shigella dysenteriae* produces a very potent cytotoxin called Shiga toxin. Nontoxigenic variants of this organism are still pathogenic but are not as virulent. The high death rates associated with toxigenic *S. dysenteriae* appear to be associated with damage done to the colonic vasculature by Shiga toxin.

Toxin may not be essential for disease

## *Ras Inhibitors and Other Toxins Affecting Host Cell Trafficking and Signal Transduction Pathways*

The A–B toxin paradigm focused on the fact that a variety of distinct toxins harbored by a variety of distinct pathogens attached the ADP-ribose moiety from NAD to a preferred target molecule, generally a G protein that bound and hydrolyzed GTP. However, the B (binding) specificity of the toxins varies considerably. Thus, seemingly identical catalytic properties of toxin molecules have different effects in a host animal because the toxin binds to a different receptor molecule in the host. For example, the most potent neurotoxins known produced by the clostridia causing botulism and tetanus target four proteins (syntaxin, VAMP/synaptobrevin 1 and 2, and snap-25) that are involved in the docking of host cell vesicles and are involved with the release of neurotransmitters. Yet, each toxin is delivered differently and preferentially binds to different cell types when introduced into humans by accidental oral ingestion or by introduction by contaminated soil. Because these toxins were recognized to be introduced into host cells and functioned intracellularly, they became a favored reagent of cell biologists to investigate the normal biology of mammalian cells.

G protein is a common target

Binding may be to multiple receptors

Some toxins, such as botulinum toxin, are used in medicine to relieve the effects of some nerve disorders. The recognition that many toxins are internalized in a membrane-bound vesicle from which the catalytically active A part has to escape into the cytoplasm led to the investigation of binding specificity within the toxin itself. In this vein, the A subunit of cholera toxin has a C-terminal motif that provides retention of the molecule in the endoplasmic reticulum; similar binding motifs are found in other toxic molecules. In recent years, the capacity of invading bacteria and other parasites to undermine the host cell biology with such exquisite sensitivity has become a hallmark of research into bacterial pathogenicity. Ten years ago, we scarcely dreamed that the study of bacterial toxins would provide such a wealth of information about human biology. The most avid medical microbiologists did not think that such a diversity of bacterial toxins were yet to be discovered. For example, a number of bacterial toxins have been recognized that modifies proteins of the Ras superfamily, particularly the Rho subfamily. Some bacteria ADP-ribosylate Rho A, B, and C at a specific asparagine residue. Others, such as the bacterium *Clostridium difficile,* a commensal that can cause severe diarrhea in patients whose flora has been

Toxins interact with cell organelles and Ras proteins suppressed by antibiotic therapy, glycosylate (add a glucose moiety) to their target and attack all members of the Rho subfamily (Rho, Rac, and CDC-42). Still others, such as the dermonecrotic toxin of the whooping cough bacillus, deamidate a glutamine residue in the Rho protein, changing it to glutamic acid, which, in the end, causes large-scale cytoskeletal rearrangements.

## *Membrane-Active Exotoxin*

While the A–B exotoxins and the toxins described thus far are, strictly speaking, intracellular toxins, a plethora of other bacterial toxins are described in the medical literature. Most of these are not well characterized, although many of them act directly on the surface of host cells to lyse or to kill them. They may facilitate penetration of host epithelial or endothelial barriers, and some toxins can kill white cells or paralyze the local immune system. Many bacteria elaborate substances that cause hemolysis of erythrocytes, and this property has been postulated to be an important virulence trait. In fact, some bacterial hemolysins are representative of general classes of bacterial exotoxins (the cytotoxins) that kill host cells by disrupting the host cell membrane. Moreover, hemolysins may liberate necessary growth factors such as iron for the invading microorganisms.

Red blood cells, white blood cells, and epithelial cells are penetrated

Among Gram-negative bacteria, a surprising number of these cytotoxins are members of a single family called the RTX (repeats in toxin) group based on a recurrent theme of a nine-amino-acid tandem duplication. RTX toxins are calcium-dependent proteins that act by creating pores in eukaryotic membranes, which may cause cellular death or at least a perturbation in host cell function. Such toxins are thought to be particularly effective against phagocytic cells. Other exotoxins contribute to the capacity of an organism to invade and spread. The lecithinase $\alpha$-toxin of *Clostridium perfringens,* for example, disrupts the membranes of a wide variety of host cells, including the leukocytes that might otherwise destroy the organism, and produces the necrotic anaerobic environment in which it can multiply.

RTX toxins create membrane pores

## *Hydrolytic Enzymes and Nontoxic Toxins of Type III Secretion Systems*

Many bacteria produce one or more enzymes that are nontoxic per se but facilitate tissue invasion or help protect the organism against the body's defense mechanisms. For example, various bacteria produce collagenase or hyaluronidase or convert serum plasminogen to plasmin, which has fibrinolytic activity. Although the evidence is not conclusive, it is reasonable to assume that these substances facilitate spread of infection. Some bacteria also produce deoxyribonuclease, elastase, and many other biologically active enzymes, but their function in the disease process or in providing nutrients for the invaders is uncertain. All are proteins and have most of the characteristics of exotoxin, except specific toxicity. Although many such factors have been thought to be involved in bacterial virulence, formal proof that they may contribute to pathogenicity has not been obtained in many cases.

Secreted proteins may facilitate other aspects of virulence

In the past 5 years there has been a growing recognition that many Gram-negative bacteria have blocks of genes called pathogenicity islands (PAIs) (see below), which are composed of a secretory pathway that delivers virulence factors into the cytoplasm of host cells. Several of these were described earlier when considering *Salmonella* invasion. The difference between these molecules and the classical bacterial toxins is that these virulence molecules are not in and of themselves toxic but they induce host cellular damage like apoptosis. These factors are described in considerable detail in the chapter that describes *Salmonella, Shigella,* and *Yersinia* (see Chapter 21).

Cellular changes are induced

## *Superantigens: Exotoxins That Interfere With the Immune Response*

It has become clear in recent years some microbial exotoxins have a direct effect on cells of the immune system and this interaction leads to many of the symptoms of disease. Thus, the enterotoxins causing staphylococcal food poisoning, the group A streptococcal exotoxin A responsible for scarlet fever, and the TSS exotoxin responsible for the staphylococcal toxic shock syndrome interact directly with the T-cell receptor. The effect of this interaction is dramatic. Cytokines such as IL-1 and TNF are produced, which leads to their familiar effects systemically and to local skin and gastrointestinal effects (depending on the toxin and its site of action). In addition, after binding to class II major histocompatibility complex (MHC) molecules on antigen-presenting cells, these exotoxins act as polyclonal stimulators of T cells so that a significant proportion of all T cells respond by dividing and releasing cytokines. This eventually leads to immunosuppression for reasons that are not totally clear. When trying to assess these findings from the standpoint of bacterial pathogenicity, it is important to divorce the disease entity seen in ill patients from the potential role of these toxins in the normal life of the microorganism.

Bind directly to T-cell receptor

Cytokines are released from large proportion of T cells

For example, staphylococcal food poisoning is an intoxication and does not involve infection by living microbes but rather the ingestion of the products produced by staphylococci in improperly handled food. The toxins that cause such food poisoning are resistant to digestive enzymes. Staphylococcal enterotoxins are also resistant to boiling, so that disease may follow ingestion of contaminated foods in which the organism has already been killed. What then is the role of the toxin in the normal biology of the microbe? Although the complete answer to this question is unknown, it seems likely that the toxins would play a role in the interaction of the microorganism with local host defenses in its preferred human niche, on the skin and the mucosal surface. Here, at the microscopic level, the capacity to neutralize the antigen-presenting cells in the microcosm of the pores of the skin is clearly more important than the induction of vast systemic symptoms. Not all staphylococci carry the enterotoxin genes. Indeed, enterotoxin genes may be carried on plasmids or bacterial viruses. Perhaps, the staphylococci that carry such "superantigens" have an advantage over their competing brethren. Such questions need to be answered at the experimental level. We must examine the determinants of bacterial pathogenicity with an eye to their role in the biology of the microbe, as well as from the view that they play an essential role in relatively rare cases of overt disease.

Ingestion of preformed toxin is a cause of food poisoning

Superantigens are not restricted simply to bacterial toxins of Gram-positive bacteria. Increasingly, they are reported as potential factors in the pathogenesis of viral infection and in a number of other bacteria. Moreover, polyclonal activation of other immune cells is seen, as in the activation of B cells by the Epstein–Barr virus. Hence, the interaction of microbial products directly with cells of the immune system that leads to immunosuppression may be a common theme of microbial pathogenicity.

## Endotoxin

In many infections caused by Gram-negative organisms, the endotoxin (see Chapter 2) of the outer membrane is a significant component of the disease process. Recall that endotoxin is a lipopolysaccharide and that the lipid portion (lipid A) is the toxic portion. The conserved polysaccharide core and the variable O-polysaccharide side chains of endotoxin are responsible for the antigenic diversity seen among enteric bacterial species. The major characteristics of endotoxin are contrasted with those of exotoxin in Table 10–3. As noted earlier, endotoxin is a major cue to the human innate defense system that bacterial multiplication is taking place in the tissues. Endotoxin in nanogram amounts causes fever in humans through release of IL-1 and TNF from macrophages. In larger amounts, whether on intact Gram-negative organisms or cell wall fragments, it produces dramatic physiologic effects associated with inflammation. These include hypotension, lowered polymorphonuclear leukocyte and platelet counts from increased margination of these cells to the walls of the small vessels, hemorrhage, and sometimes disseminated intravascular coagulation from activation of clotting factors. Rapid and irreversible shock may follow passage of endotoxin into the bloodstream. This syndrome is seen when materials that have become heavily contaminated are injected intravenously or when a severe local infection leads to massive bacteremia. The role of endotoxin in more chronic disease processes is less clear, but some manifestations of typhoid fever and meningococcal septicemia, for example, are fully compatible with the known effects of endotoxin in humans. It should be noted that endotoxins are considerably less active than many

Located in Gram-negative outer membrane

Lipid A is most toxic

Shock syndrome may be fatal

TABLE 10–3

**Differential Characteristics of Endotoxins and Exotoxins**

| Characteristic | Endotoxins | Exotoxins |
|---|---|---|
| Chemical nature | Lipopolysaccharide (lipid A component) | Protein |
| Part of Gram-negative cell outer membrane | Yes | No |
| Most from Gram-positive bacteria | No | Yes |
| Usually extracellular | No | Yes |
| Phage or plasmid coded | No | Many |
| Antigenic | Weakly | Yes |
| Can be converted to toxoid | No | Many |
| Neutralized by antibody | Weakly | Yes |
| Differing pharmacologic specificities | No | Yes |
| Stable to boiling[a] | Yes | No |

[a] Enterotoxin of *Staphylococcus aureus* withstands boiling.

exotoxins, incompletely neutralized by antibody against their carbohydrate component, and stable even to autoclaving. The latter characteristic is important, because materials for intravenous administration that have become contaminated with Gram-negative organisms are not detoxified by sterilization.

Gram-positive bacteria do not contain endotoxin but they release peptidoglycan fragments and other cell wall determinants that act to "alarm" the host to the presence of bacteria in the tissues. The same cytokines are released and the same physiologic cascade is seen.

## AVOIDING THE HOST IMMUNE SYSTEM

The host immune system evolved in large part because of the selective pressure of microbial attack. To be successful, microbial pathogens must escape this system at least long enough to be transmitted to a new susceptible host or to take up residence within the host in a way that is compatible with mutual coexistence.

### Serum Resistance

Many bacteria that come into contact with human complement can be destroyed by opsonization or by direct lysis of the bacterial membrane by complement complexes. Some can avoid this fate by a process called serum resistance. Pathogenic *Salmonella* possess a lipopolysaccharide inhibiting the C5b–9 complement complex from attacking the hydrophobic domains of the bacterial outer membrane. Other bacteria employ different mechanisms, but the end result is the same. These organisms can persist in an environment that is rapidly lethal for nonpathogens.

Complement interference allows persistence

### Antiphagocytic Activity

A fundamental requirement for many pathogenic bacteria is escape from phagocytosis by macrophages and polymorphonuclear leukocytes. It seems likely that the ability to avoid phagocytosis was an early necessity for microorganisms following the evolution of predatory protozoans. Some bacteria such as the causative agent of Legionnaires' disease, *Legionella pneumophila,* learned how to replicate in free-living amoebae following phagocytosis and used them as part of their life cycle. *Legionella* uses similar mechanisms to outwit human macrophages. In this one example, it can be seen that pathogenicity in some microorganisms evolved from a very early time in their development.

Avoiding phagocytosis is big advantage

The most common bacterial means to avoid phagocytosis is an antiphagocytic capsule. The significance of the bacterial capsule can hardly be overemphasized. Almost all principal pathogens that cause pneumonia and meningitis have antiphagocytic polysaccharide capsules. Nonencapsulated variants of these organisms are usually avirulent. In many cases, it has been found that the capsule of pathogens prevents complement deposition on the bacterial cell surface. Thus, the capsule prevents nonimmune opsonization and confers resistance to phagocytosis. As noted earlier, along with encapsulation, a common factor of many organisms that cause pneumonia and meningitis is the elaboration of an enzyme that specifically cleaves human IgA1 molecules. IgA proteases are found in the pathogenic *Neisseria, Haemophilus influenzae* type b (Hib), and *Streptococcus pneumoniae.* The combination of a capsule to avoid opsonization and/or an enzyme that cleaves an important class of secretory antibody is a potent stratagem to avoid phagocytosis.

Capsules block complement deposition

The group A streptococcal M protein is another example of a bacterial surface product employed by the organism to escape opsonization and phagocytosis. In part, this is a reflection of the ability of M protein to bind fibrinogen and its breakdown product fibrin to the bacterial surface. This sterically hinders complement access and prevents opsonization. There are many other examples. The principle is clear. If microorganisms can inhibit phagocytosis, they can often gain the upper hand long enough to replicate sufficiently to establish themselves in the host or become transmitted to a new host. It is important to understand that these encapsulated pathogens are often carried asymptomatically in the normal flora (see Table 9–1). The capsule is important for the organism to establish itself in the nasopharynx.

Surface proteins block complement and bind fibrinogen

The host responds to its initial encounter with the encapsulated organism by elaborating anticapsular antibodies that opsonize and permit efficient phagocytosis and destruction of the microorganism in subsequent encounters. Thus, the initial interaction between an encapsulated microbe and its host usually has two outcomes. First, the host becomes asymptomatically colonized, and, second, the colonization is an immunizing event for the host. The host is protected against serious systemic infection by the organism, but this immunity may not affect the capacity of the organism to live happily on a mucosal surface. Epidemiologic investigations show that serious disease caused by encapsulated pathogens when it occurs does so shortly after a susceptible individual encounters the microorganism for the first time. This scenario contrasts with the idea that carriers of microorganisms come down with the disease at some time in the future. If a microorganism meets a host with a compromised immune system or some short-term deficit in its defense systems, then the organism's capacity for replication can overwhelm the host defense mechanisms and cause serious disease. Once colonization and immunity have been established, the steady state is a satisfactory host–parasite relationship. For example, the outcome of encounters with *Neisseria meningitidis* in military recruits followed for colonization and anticapsular antibody throughout training camp has been demonstrated. Disease developed only in those entering the camp lacking both specific antibody and nasopharyngeal colonization with the *N. meningitidis* serogroup responsible for a subsequent meningitis outbreak. Unaffected recruits either had a "successful" encounter followed by development of antibody or already had protective antibody, presumably from a similar experience earlier in life.

Opsonizing antibody reverses effect of capsule

Disease occurs in period of absent immune response

## Cutting Lines of Communication

Pathogens such as *Yersinia* and *Salmonella* have evolved means to neutralize phagocytes directly by using the equivalent of eukaryotic signal transduction molecules. Pathogenic *Yersinia* synthesize tyrosine phosphatase molecules and serine kinase molecules and introduce them into the cytoplasm of macrophages, which leads to a complete loss in the capacity of these cells either to phagocytose microorganisms or to signal other components of the host immune system by cytokine release. Likewise, both *Salmonella* and *Yersinia* inject bacterial proteins into the cytoplasm of host cells that directly induce apoptosis or disrupt cellular function. As noted earlier, microbial mimicry can have the same effect by concealing the microorganism under a shroud of host proteins; however, the strategy of directly interfering with host cell function by use of an alternative enzyme

Secreted proteins alter phagocyte function or modifying and activating existing host cell effectors has been discovered to be a more common pathogenic strategy for the invading microbe than previously realized. This helps resolve the mystery that bacteria not known to produce toxins nonetheless cause cellular toxicity.

### Antigenic Variation

Another method by which microorganisms avoid host immune responses is by varying surface antigens. *N. gonorrhoeae* displays an endless array of pili and outer membrane proteins to the host immune system. The organism has learned to preserve its binding specificity but to vary endlessly the molecular scaffolding on which the functional units are placed. The host "sees" a bewildering array of new epitopes, whereas the critical regions of the molecule remain hidden from immune surveillance. Of course, among the viruses, antigenic variation is also a common theme; the best known example is the influenza virus. It is instructive that in both the bacterial example and the viral example, recombination mechanisms act to bring together novel sequences of genetic material. A number of microorganisms known for their antigenic diversity such as those of the genus *Borrelia,* which causes relapsing fever, and the group A streptococci also use homologous recombination of DNA from repeated sequences to generate the diversity in size and sequence observed in their principal immunodominant antigens.

Alteration in surface epitopes confounds immune surveillance

## INADVERTENT TISSUE DAMAGE FROM IMMUNE REACTIONS DIRECTED AGAINST INVADING BACTERIA

Tissue damage and the manifestations of disease may also result from interaction between the host's immune mechanisms and the invading organism or its products. Reactions between high concentrations of antibody, soluble microbial antigens, and complement can deposit immune complexes in tissues and cause acute inflammatory reactions and immune complex disease. In poststreptococcal acute glomerulonephritis, for example, the complexes are sequestered in the glomeruli of the kidney, with serious interference in renal function from the resulting tissue reaction. Sometimes, antibody produced against microbial antigens can cross-react with certain host tissues and initiate an autoimmune process. Such cross-reaction is almost certainly the explanation for poststreptococcal rheumatic fever, and it may be involved in some of the lesions of tertiary syphilis. Some viruses have been shown to have small peptide sequences that are occasionally shared by host tissues. Thus, a virus-induced immune response may also generate antibodies that react with shared determinants on host cells, such as in the heart.

Immune reactions may injure or cross-react with host tissue

In some other infections, the pathologic and clinical features are due largely to delayed-type hypersensitivity reactions to the organism or its products. Such reactions are particularly significant in tuberculosis and other mycobacterial infections. The mycobacteria possess no significant toxins, and in the absence of delayed hypersensitivity, their multiplication elicits little more than a mild inflammatory response. The development of delayed-type, cell-mediated hypersensitivity to their major proteins leads to dramatic pathologic manifestations, which in tuberculosis comprise a chronic granulomatous response around infected foci with massive infiltration of macrophages and lymphocytes followed by central devascularization and necrosis. Rupture of a necrotic area into a bronchus leads to the typical pulmonary cavity of the disease; rupture into a blood vessel can produce extensive dissemination or massive bleeding from the lung. Injection of tuberculoprotein into an animal with an established tuberculous lesion can lead to acute exacerbation and sometimes death. Thus, the body's defense mechanisms are themselves contributing to the severity of the disease process.

Continued delayed-type hypersensitivity causes injury

Granuloma is typical manifestation

These examples illustrate processes that are probably involved to varying degrees in the pathology and course of most infections. Immune reactions are essential to the control of infectious diseases; however, they are potentially damaging to the host, particularly when large amounts of antigens are involved and the host response is unusually active. It is likely that some pathogenic bacteria have deliberately modified the nature of the host immune response so that the effects are directed away from direct antimicrobial factors.

By the same token, the misdirected immune response may provide a needed niche for the invading microbe to complete its mission of survival.

## DISEASE AND TRANSMISSIBILITY

Host survival facilitates pathogen survival

Lethal disease is probably an inadvertent and even unfavorable outcome of infection from the standpoint of a microorganism. Pathogens that are highly adapted to their host usually spare the majority of their victims. In many cases, it is to the advantage of the microbe to cause some degree of illness that may aid its transmission. In other cases, the interplay between the microbe and the host is subclinical resolution; there may be damage but no disease. Indeed, many of the most severe infectious diseases occur when a microorganism adapted to a nonhuman environment finds itself inadvertently in a human host. The probability of disease is a reflection of the microbial design to live and multiply within a host balanced against the host's capacity to control and limit bacterial proliferation. For certain microorganisms, such as *Streptococcus pyogenes,* contact with susceptible hosts that possess normal host defense systems renders a certain proportion clinically ill. In contrast, normal individuals usually shrug off *Proteus* and *Serratia* species. How different the outcome of this interaction when the host is compromised!

For microbes exclusively adapted to humans, transmissibility is the key to continued survival. For many organisms, this entails microbial persistence in the host and in the environment. A stable pathogen population must retain its viability outside of its preferred niche and still be capable of infection when it next encounters a susceptible host. We are still rather ignorant of the microbial factors at play that ensure their transmissibility from host to host. These conditions are difficult to recapitulate experimentally. However, the use of bacteria carrying sensitive reporter molecules will likely permit a better view of transmissibility.

## COROLLARIES OF MICROBIAL PATHOGENICITY

As noted, all parasitic microorganisms need to enter a host, find a unique niche, overcome local defenses, replicate, and be transmitted to a new host. Other factors have become apparent because of these pathogenic attributes. Some are more applicable to bacteria and fungi than to viruses and the larger parasites. The general principles are likely to be true for all pathogens.

Successful pathogens have created a balanced adaptation to the host

1. **Pathogenic microorganisms adapt to changes in the host's biological and social behavior.** Imagine the profound changes in the host–parasite relationship that must have occurred when humanoids began to live in communities and began to husband animals. The older diseases such as tuberculosis remained, but the increase in population density meant that "new" epidemic diseases could evolve. In recent times, we have seen new diseases emerge. Diseases such as TSS, Legionnaires' disease, and nosocomial infections are a reflection, in part, of human progress. We need to remind ourselves that we live in a balanced relationship with microorganisms on this planet. Microorganisms will take advantage of any selective benefit that is made available to them to replicate and to establish themselves in a new niche. The advent of the birth control pill and the replacement of barrier contraception led to an enormous increase in sexually transmitted diseases. As humans increasingly impinge on other forms of life that have been largely isolated from human populations, there has been an increase in "new" infectious diseases such as Lyme disease, and quite probably, AIDS. As we have become more efficient at food production and mass global distribution, there has been an increase, rather than a decrease, in food-borne infection and disease. One need no longer go to an esoteric place in the world to acquire traveler's diarrhea, it can be readily acquired on imported food now at the corner food market!
2. **Pathogens are clonal.** Bacteria are haploid, as are viruses and some fungi. Consequently, there cannot be a helter-skelter amalgam of genes brought about by promiscuous genetic exchange. If this were so, there would be no bacterial specialization and all would possess a consensus chromosomal sequence. Thus, most bacteria (and

TABLE 10–4

**Proportion of Certain Infectious Diseases Caused by Common Bacterial Clonal Types**

| Species | Total Number of Clonal Types Identified | Number of Clonal Types Commonly Isolated from Cases of Disease | Percentage of Disease Due to Common Clonal Types |
|---|---|---|---|
| *Bordetella pertussis* | 2 | 2 | 100 |
| *Haemophilus influenzae* type b | | | |
| North America | 104 | 6 | 81 |
| Europe | 60 | 3 | 78 |
| *Legionella pneumophila* | | | |
| Global | 50 | 5 | 52 |
| Wadsworth VA Hospital | 10 | 1 | 86 |
| *Shigella sonnei* | 1 | 1 | 100 |

*Modified from Mandell GL, Bennett JE, Dolin R.* Principles and Practice of Infectious Diseases, *5th ed. New York: Churchill Livingstone; 2002, with permission.*

viruses) have some degree of built-in reproductive isolation, except for members of their own or very closely related species (members of the same gene pool). In this way, diversity within the species through mutation can be maximized (usually by transformation or transduction), while conserving useful gene sequences. The end result of husbanding of important genes during evolution is that at any given time in the world, many bacterial and viral pathogens are representatives of a single or, more often, a relatively few clonal types that have become widespread for the (evolutionary) moment. Thus, all the strains of the typhoid bacillus that have been studied since humans learned to culture them belong to two basic clonal types (Table 10–4). When microbes establish a unique niche, they protect their selective advantage.

Useful genes are preserved by clonality

However, the bacterial gene pool must be expanded. Indeed, how could microorganisms have become pathogens in the first place or adapt to new potential niches? Bacteria have remarkable ways of expanding their genetic diversity, but they do so in a way that is consistent with their haploid lifestyle. From this corollary follows the next.

3. **Pathogens often carry essential virulence determinants on mobile genetic elements.** It is now well established that many of the essential determinants of pathogenicity are actually replicated as part of an extrachromosomal element or as additions to the bacterial chromosome (Table 10–5). If haploid organisms must limit their genetic interactions to preserve their individuality, it is not surprising that new genes with important new attributes are found on genetic elements that do not disrupt the organization of the bacterial chromosome. The interchange of plasmids and bacterial viruses among bacteria, coupled with transpositional (illegitimate) recombination between the extrachromosomal element and the chromosome, provides a means for microorganisms to exploit new genes in a haploid world. It is of some note that pathogenic determinants not found associated with a plasmid or a phage are often seen as duplicated genes or associated with transposon-like structures.

Virulence determinants are often extrachromosomal and transmissible

While it was clear for some time that mobile genetic elements played an essential role in the evolution of pathogenicity, only recently, with the advent of new DNA sequencing methods, have we learned that large blocks of genes found on the bacterial chromosome are associated with pathogenicity. These blocks of genes have been given the name pathogenicity islands (PAIs) to describe unique chromosomal regions found exclusively associated with virulence. It is now generally believed that parts of a plasmid associated with virulence are likely PAIs as well. PAIs most often occupy large genomic areas of 10 to 200 or more kilobases. However, certain bacterial strains also carry insertions of smaller

Multiple pathogenicity genes are present in PAIs

TABLE 10–5

**Examples of Plasmid and Phage-Encoded Virulence Determinants**

| Organism | Virulence Factor | Biological Function |
|---|---|---|
| **Plasmid-encoded** | | |
| Enterotoxigenic *Escherichia coli* | Heat-labile, heat-stable enterotoxins (LT, ST) | Activation of adenyl/guanylcyclase in the small bowel, which leads to diarrhea |
| | CFA/I and CFA/II | Adherence/colonization factors |
| *Salmonella* spp. | Serum resistance and intracellular survival | Invasion of reticuloendothelial system |
| *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 adenylcyclase activity |
| *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* | Neurotoxin | Blocks synaptic acetylcholine release, which leads to flaccid paralysis |
| Enterohemorrhagic *E. coli* | Shiga-like toxin | Inhibition of eukaryotic protein synthesis |

*Modified from Mandell GL, Bennett JE, Dolin R.* Principles and Practice of Infectious Diseases, *5th ed. New York: Churchill Livingstone; 2002, with permission.*

pieces of DNA with the attributes of PAIs, but they are only 1 to 10 kilobases in size and are referred to by some as pathogenicity islets. All of the available data are consistent with the idea that horizontal gene transfer likely is mediated by phage or plasmids acquired these large (and small) DNA sequences. However, the PAIs described thus far are not mobile in themselves. There is an eerie quality about the composition of many PAIs in the sense that they have a very different guanine + cytosine content and codon usage as compared to the rest of the genome. The fact PAIs are so often associated with tRNA genes suggests that gene transfer from a foreign species is the likely origin. (In many prokaryotic and eukaryotic species, tRNA genes often act as the site of integration of foreign DNA.) Many PAIs have strikingly similar homologs in bacteria that are pathogenic for plants and animals and range from obligate intracellular parasites such as *Chlamydia* to free-living environmental opportunistic pathogens such as *Pseudomonas aeruginosa.*

PAIs are organized blocks of genes that appear to come from an unrelated organism

In a bacterial genus that contains both pathogenic and nonpathogenic species, the attributes of pathogenicity are encoded on sequences that do not have any counterpart in the nonpathogen. It seems unlikely that pathogenicity arose as a result of long adaptation of an initially nonpathogenic organism to a more parasitic, host-dependent lifestyle. It is more likely that organisms inherited new gene sequences, often in a large block, that provided them with the capacity to establish themselves more efficiently in a host or to exploit some new niche within the host.

TABLE 10–6

**Examples of Bacterial Virulence Regulatory Systems**

| Organism | Regulatory Gene(s) | Environmental Stimuli | Regulated Functions |
|---|---|---|---|
| *Escherichia coli* | *drd*X | Temperature | Pyelonephritis-associated pili |
| | *fur* | Iron concentration | Shiga-like toxin, siderophores |
| *Bordetella pertussis* | *bvg*AS | Temperature, ionic conditions, nicotinic acid | Pertussis toxin, filamentous hemagglutinin, adenylate cyclase, others |
| *Vibrio cholerae* | *tox*R | Temperature, osmolarity, pH, amino acids | Cholera toxin, pili, outer membrane proteins |
| *Yersinia* spp. | *lcr* loci | Temperature, calcium | Outer membrane proteins |
| | *vir*F | Temperature | Adherence, invasiveness |
| *Shigella* spp. | *vir*R | Temperature | Invasiveness |
| *Salmonella typhimurium* | *pag* genes | pH | Virulence, macrophage survival |
| *Staphylococcus aureus* | *agr* | pH | $\alpha$-, $\beta$-hemolysins; toxic shock syndrome toxin 1, protein A |

*Modified from Mandell GL, Bennett JE, Dolin R.* Principles and Practice of Infectious Diseases, *5th ed. New York: Churchill Livingstone; 2002, with permission.*

4. **Bacteria and other pathogens use elaborate means to modulate their free-living life from their parasitic life.** Bacteria, fungi, and larger parasites have evolved signal transduction networks using environmental clues such as temperature, iron concentration, and calcium flux to turn on genes important for pathogenicity (Table 10–6). It was puzzling to consider how a microorganism that makes potent toxins in the laboratory could possibly spare any host it infected. It became clearer when we learned that toxin biosynthesis by the microbe is tightly regulated together with other genes to be activated only in particular sets of circumstances. Only in selective circumstances are genes involved in pathogenicity used and then often sparingly. The organism's reaction to the host need only be sufficient to establish itself and replicate.

Regulatory systems sense temperature and ions

## CONCLUSION

Host-parasite interactions are wonderfully complex and have evolved in a manner that has tended to produce a more balanced state of parasitism between well-established species and the microorganisms with which they frequently come into contact. In this chapter, the components of these interactions have been discussed separately, but it is important to recognize the dynamic and shifting nature of their role in determining the course and outcome of an infection. As you review the microbial tactics for survival and the ensuing host response to acute infection and its consequences, it is important to see that systemic symptoms of many viral and bacterial infections—fever, malaise, and anorexia—are the same because they reflect a basic innate host response to a foreign intruder. The diversity of mechanisms by which a host controls infection can be particularly appreciated if one recognizes that they are all intimately interrelated. Many of the infected patients you will aid during your career will not have inherited defects in their host defense matrix, but they will have disease-associated deficiencies. Increasingly, cytotoxic chemotherapy, radiotherapy, and other forms of medical intervention bring about physician-induced deficits in their innate and adaptive immune systems. For example, because of their tumors or treatment, cancer patients often have a variety of interrelated defects and mucosal disruptions increasing the risk of infection. The single most important of these is neutropenia (usually defined as an absolute granulocyte count of 500/mm$^3$ or less). It is no wonder that the presenting symptom in tumors of the bowel may be sepsis or bacterial endocarditis. During the natural progression of malignancy and as a consequence of its current therapy, infection remains as the major cause of morbidity and mortality.

The above examples suffice, but the significant point is the importance of normal defense mechanisms. It is more important to preserve and augment the normal host defenses of a patient in many circumstances than to use the newest "wonder drug." We can also be fully confident that an understanding of the molecular basis of microbial pathogenesis will provide considerable information about the biology of the pathogens, the host-parasite relationship, human (and other) host-specific defense mechanisms, and, ultimately, ways to prevent infection and disease. Finally, from this brief overview of the properties of pathogenic bacteria, it is important to never underestimate the capacity of these small creatures to persist and survive in human society.

## ADDITIONAL READING

Salyers AA, Whitt DD. *Bacterial Pathogenesis: A Molecular Approach.* 2nd ed. Washington, D.C.: American Society for Microbiology; 2002. This modern text beautifully discusses topics from the clinical to the molecular level.

# PART IV

# SPREAD AND CONTROL OF INFECTION

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CHAPTER 11

# Sterilization and Disinfection

KENNETH J. RYAN

From the time of debates about the germ theory of disease, killing microbes before they reach patients has been a major strategy for preventing infection. In fact, Ignaz Semmelweiss successfully applied disinfection principles decades before bacteria were first isolated (see Chapter 72). This chapter discusses the most important methods used for this purpose in modern medical practice. Understanding how they work is of increasing importance in an environment that includes immunocompromised patients, transplantation, indwelling devices, and acquired immunodeficiency syndrome (AIDS).

## DEFINITIONS

**Death/killing** as it relates to microbial organisms is defined in terms of how we detect them in culture. Operationally, it is a loss of ability to multiply under any known conditions. This is complicated by the fact that organisms that appear to be irreversibly inactivated may sometimes recover when appropriately treated. For example, ultraviolet (UV) irradiation of bacteria can result in the formation of thymine dimers in the DNA with loss of ability to replicate. A period of exposure to visible light may then activate an enzyme that breaks the dimers and restores viability by a process known as photoreactivation. Mechanisms also exist for repair of the damage without light. Such considerations are of great significance in the preparation of safe vaccines from inactivated virulent organisms.

Absence of growth does not necessarily indicate sterility

**Sterilization** is complete killing, or removal, of all living organisms from a particular location or material. It can be accomplished by incineration, nondestructive heat treatment, certain gases, exposure to ionizing radiation, some liquid chemicals, and filtration.

Sterilization is killing of all living forms

**Pasteurization** is the use of heat at a temperature sufficient to inactivate important pathogenic organisms in liquids such as water or milk but at a temperature below that needed to ensure sterilization. For example, heating milk at a temperature of 74°C for 3 to 5 seconds or 62°C for 30 minutes kills the vegetative forms of most pathogenic bacteria that may be present without altering its quality. Obviously, spores are not killed at these temperatures.

Pasteurization uses heat to kill vegetative forms of bacteria

**Disinfection** is the destruction of pathogenic microorganisms by processes that fail to meet the criteria for sterilization. Pasteurization is a form of disinfection, but the term is most commonly applied to the use of liquid chemical agents known as disinfectants, which usually have some degree of selectivity. Bacterial spores, organisms with waxy coats (eg, mycobacteria), and some viruses may show considerable resistance to the common disinfectants. **Antiseptics** are disinfectant agents that can be used on body surfaces such as the skin or vaginal tract to reduce the numbers of normal flora and pathogenic

Disinfection uses chemical agents to kill pathogens with varying efficiency

Spores are particularly resistant

contaminants. They have lower toxicity than disinfectants used environmentally but are usually less active in killing vegetative organisms. **Sanitization** is a less precise term with a meaning somewhere between disinfection and cleanliness. It is used primarily in housekeeping and food preparation contexts.

Asepsis applies sterilization and disinfection to create a protective environment

**Asepsis** describes processes designed to prevent microorganisms from reaching a protected environment. It is applied in many procedures used in the operating room, in the preparation of therapeutic agents, and in technical manipulations in the microbiology laboratory. An essential component of aseptic techniques is the sterilization of all materials and equipment used. Asepsis is more fully discussed in Chapter 72.

## MICROBIAL KILLING

Bacterial killing follows exponential kinetics

Killing of bacteria by heat, radiation, or chemicals is usually exponential with time; that is, a fixed proportion of survivors are killed during each time increment. Thus, if 90% of a population of bacteria are killed during each 5 minutes of exposure to a weak solution of a disinfectant, a starting population of $10^6$/mL is reduced to $10^5$/mL after 5 minutes, to $10^3$/mL after 15 minutes, and theoretically to 1 organism ($10^0$)/mL after 30 minutes. Exponential killing corresponds to a first-order reaction or a "single-hit" hypothesis in which the lethal change involves a single target in the organism, and the probability of this change is constant with time. Thus, plots of the logarithm of the number of survivors against time are linear (Fig 11–1A); however, the slope of the curve varies with the effectiveness of the killing process, which is influenced by the nature of the organism, lethal agent, concentration (in the case of disinfectants), and temperature. In general, the rate of killing increases exponentially with arithmetic increases in temperature or in concentrations of disinfectant.

Achieving sterility is a matter of probability

An important consequence of exponential killing with most sterilization processes is that sterility is not an absolute term, but must be expressed as a probability. Thus, to continue the example given previously, the chance of a single survivor in 1 mL is theoretically $10^{-1}$ after 35 minutes. If a chance of $10^{-9}$ were the maximum acceptable risk for a single surviving organism in a 1-mL sample (eg, of a therapeutic agent), the procedure would require continuation for a total of 75 minutes.

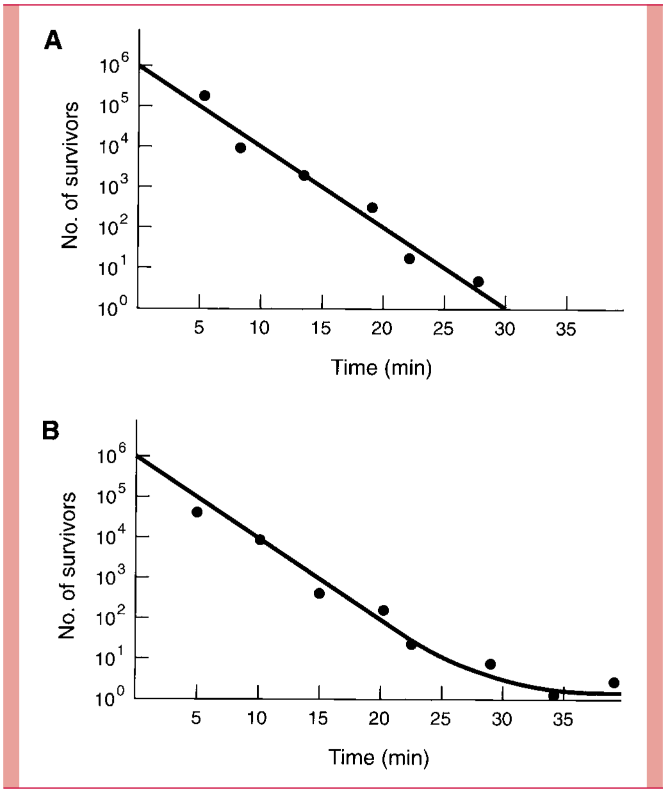


FIGURE 11–1
Kinetics of bacterial killing. **A.** Exponential killing is shown as a function of population size and time. **B.** Deviation from linearity, as with a mixed population, extends the time.

A simple single-hit curve often does not express the kinetics of killing adequately. In the case of some bacterial endospores, a brief period (activation) may elapse before exponential killing by heat begins. If multiple targets are involved, the experimental curve will deviate from linearity. More significant is the fact that microbial populations may include a small proportion of more resistant mutants or of organisms in a physiologic state that confers greater resistance to inactivation. In these cases, the later stages of the curve are flattened (Fig 11–1B), and extrapolations from the exponential phase of killing may seriously underestimate the time needed for a high probability of achieving complete sterility. In practice, materials that come into contact with tissues are sterilized under conditions that allow a very wide margin of safety, and the effectiveness of inactivation of organisms in vaccines is tested directly with large volumes and multiple samples before a product is made available for use.

Heterogeneous microbial subpopulations may extend the killing kinetics

## STERILIZATION

The availability of reliable methods of sterilization has made possible the major developments in surgery and intrusive medical techniques that have helped to revolutionize medicine over the past century. Furthermore, sterilization procedures form the basis of many food preservation procedures, particularly in the canning industry. The various modes of sterilization described in the text are summarized in Table 11–1.

TABLE 11–1

### Methods of Disinfection and Sterilization

| Method | Activity Level | Spectrum | Uses/Comments |
|---|---|---|---|
| **Heat** | | | |
| Autoclave | Sterilizing | All | General |
| Boiling | High | Most pathogens, some spores | General |
| Pasteurization | Intermediate | Vegetative bacteria | Beverages, plastic hospital equipment |
| **Ethylene oxide gas** | Sterilizing | All | Potentially explosive; aeration required |
| **Radiation** | | | |
| Ultraviolet | Sterilizing | All | Poor penetration |
| Ionizing | Sterilizing | All | General, food |
| **Chemicals** | | | |
| Alcohol | Intermediate | Vegetative bacteria, fungi, some viruses | |
| Hydrogen peroxide | High | Viruses, vegetative bacteria, fungi | Contact lenses; inactivated by organic matter |
| Chlorine | High | Viruses, vegetative bacteria, fungi | Water; inactivated by organic matter |
| Iodophors | Intermediate | Viruses, vegetative bacteria,[a] fungi | Skin disinfection; inactivated by organic matter |
| Phenolics | Intermediate | Some viruses, vegetative bacteria, fungi | Handwashing |
| Glutaraldehyde | High | All | Endoscopes, other equipment |
| Quaternary ammonium compounds | Low | Most bacteria and fungi, lipophilic viruses | General cleaning; inactivated by organic matter |

[a]Variable results with *Mycobacterium tuberculosis*.

## Heat

Incineration is rapid and effective

Dry heat requires 160°C for 2 hours to kill

The simplest method of sterilization is to expose the surface to be sterilized to a naked flame, as is done with the wire loop used in microbiology laboratories. It can be used equally effectively for emergency sterilization of a knife blade or a needle. Of course, disposable material is rapidly and effectively decontaminated by incineration. Carbonization of organic material and destruction of microorganisms, including spores, occur after exposure to dry heat of 160°C for 2 hours in a sterilizing oven. This method is applicable to metals, glassware, and some heat-resistant oils and waxes that are immiscible in water and cannot, therefore, be sterilized in the autoclave. A major use of the dry heat sterilizing oven is in preparation of laboratory glassware.

Moisture allows for rapid denaturation of protein

Boiling water fails to kill bacterial spores

Moist heat in the form of water or steam is far more rapid and effective in sterilization than dry heat, because reactive water molecules denature protein irreversibly by disrupting hydrogen bonds between peptide groups at relatively low temperatures. Most vegetative bacteria of importance in human disease are killed within a few minutes at 70°C or less, although many bacterial spores (see Chapter 2) can resist boiling for prolonged periods. For applications requiring sterility the use of boiling water has been replaced by the autoclave, which when properly used ensures sterility by killing all forms of microorganisms.

Autoclave creates increased temperature of steam under pressure

Steam displaces air from the autoclave

Killing rate increases logarithmically with arithmetic increase in temperature

In effect, the **autoclave** is a sophisticated pressure cooker (Fig 11–2). In its simplest form, it consists of a chamber in which the air can be replaced with pure saturated steam under pressure. Air is removed either by evacuation of the chamber before filling it with steam or by displacement through a valve at the bottom of the autoclave, which remains open until all air has drained out. The latter, which is termed a **downward displacement autoclave,** capitalizes on the heaviness of air compared with saturated steam. When the air has been removed, the temperature in the chamber is proportional to the pressure of the steam; autoclaves are usually operated at 121°C, which is achieved with a pressure of 15 pounds per square inch. Under these conditions, spores directly exposed are killed in less than 5 minutes, although the normal sterilization time is 10 to 15 minutes to account for variation in the ability of steam to penetrate different materials and to allow a wide margin of safety. The velocity of killing increases logarithmically with arithmetic increases in temperature, so a steam temperature of 121°C is vastly more effective than 100°C. For example, the spores of *Clostridium botulinum,* the cause of botulism, may survive 5 hours of boiling, but can be killed in 4 minutes at 121°C in the autoclave.

Condensation and latent heat increase effectiveness of autoclave

The use of saturated steam in the autoclave has other advantages. Latent heat equivalent to 539 cal/g condensed steam is immediately liberated on condensation on the cooler surfaces of the load to be sterilized. The temperature of the load is thus raised very rapidly to that of the steam. Condensation also permits rapid steam penetration of porous materials such as surgical drapes by producing a relative negative pressure at the surface, which allows more steam to enter immediately. Autoclaves can thus be used for sterilizing any

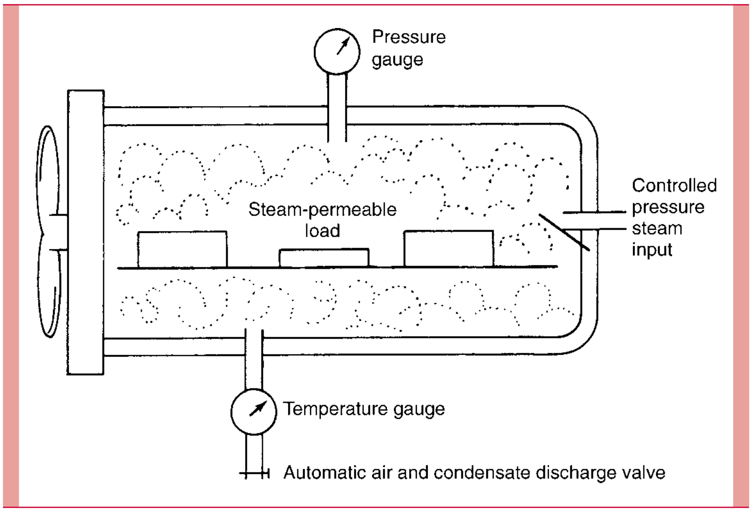


FIGURE 11–2
Simple form of downward displacement autoclave.

materials that are not damaged by heat and moisture, such as heat-stable liquids, swabs, most instruments, culture media, and rubber gloves.

It is essential that those who use autoclaves understand the principles involved. Their effectiveness depends on absence of air, pure saturated steam, and access of steam to the material to be sterilized. Pressure per se plays no role in sterilization other than to ensure the increased temperature of the steam. Failure can result from attempting to sterilize the interior of materials that are impermeable to steam or the contents of sealed containers. Under these conditions, a dry heat temperature of 121°C is obtained, which may be insufficient to kill even vegetative organisms. Large volumes of liquids require longer sterilization times than normal loads, because their temperature must reach 121°C before timing begins. When sealed containers of liquids are sterilized, it is essential that the autoclave cool without being opened or evacuated; otherwise, the containers may explode as the external pressure falls in relation to that within.

Access of pure saturated steam is required for sterilization

Impermeable or large volume materials present special problems

**"Flash" autoclaves,** which are widely used in operating rooms, often use saturated steam at a temperature of 134°C for 3 minutes. Air and steam are removed mechanically before and after the sterilization cycle so that metal instruments may be available rapidly. Quality control of autoclaves depends primarily on ensuring that the appropriate temperature for the pressure used is achieved and that packing and timing are correct. Biological and chemical indicators of the correct conditions are available and are inserted from time to time in the loads.

Flash autoclaves use 134°C for 3 minutes

## Gas

A number of articles, particularly certain plastics and lensed instruments that are damaged or destroyed by autoclaving, can be sterilized with gases. **Ethylene oxide** is an inflammable and potentially explosive gas. It is an alkylating agent that inactivates microorganisms by replacing labile hydrogen atoms on hydroxyl, carboxy, or sulfhydryl groups, particularly of guanine and adenine in DNA. Ethylene oxide sterilizers resemble autoclaves and expose the load to 10% ethylene oxide in carbon dioxide at 50 to 60°C under controlled conditions of humidity. Exposure times are usually about 4 to 6 hours and must be followed by a prolonged period of aeration to allow the gas to diffuse out of substances that have absorbed it. Aeration is essential, because absorbed gas can cause damage to tissues or skin. Ethylene oxide is a mutagen, and special precautions are now taken to ensure that it is properly vented outside of working spaces. Used under properly controlled conditions, ethylene oxide is an effective sterilizing agent for heat-labile devices such as artificial heart valves that cannot be treated at the temperature of the autoclave. Other alkylating such as **formaldehyde** vapor can be used without pressure to decontaminate larger areas such as rooms, and oxidizing agents (hydrogen peroxide, ozone) have selective use.

Ethylene oxide sterilization is used for heat-labile materials

Aeration needed after ethylene oxide sterilization

Formaldehyde and oxidizing agents are useful in sterilization

## Ultraviolet Light and Ionizing Radiation

**Ultraviolet (UV) light** in the wavelength range 240 to 280 nm is absorbed by nucleic acids and causes genetic damage, including the formation of the thymine dimers discussed previously. The practical value of UV sterilization is limited by its poor ability to penetrate. Apart from the experimental use of UV light as a mutagen, its main application has been in irradiation of air in the vicinity of critical hospital sites and as an aid in the decontamination of laboratory facilities used for handling particularly hazardous organisms. In these situations, single exposed organisms are rapidly inactivated. It must be remembered that UV light can cause skin and eye damage, and workers exposed to it must be appropriately protected.

UV light causes direct damage to DNA

Use of UV light is limited by penetration and safety

**Ionizing radiation** carries far greater energy than UV light. It, too, causes direct damage to DNA and produces toxic free radicals and hydrogen peroxide from water within the microbial cells. Cathode rays and gamma rays from cobalt-60 are widely used in industrial processes, including the sterilization of many disposable surgical supplies such as gloves, plastic syringes, specimen containers, some foodstuffs, and the like, because they can be packaged before exposure to the penetrating radiation. Ionizing irradiation does not always result in the physical disintegration of killed microbes. As a result, plasticware sterilized in this way may carry significant numbers of dead but stainable

Ionizing radiation damages DNA

Use for surgical supplies, food

Killed organisms may remain morphologically intact and stainable

bacteria. This has occasionally caused confusion when it has involved containers used to collect normally sterile body fluids such as cerebrospinal fluid. The dead bacterial bodies may produce a "false-positive" Gram-stained smear and result in inappropriate administration of antibiotics. Recent foodborne outbreaks (*Escherichia coli*) and bioterrorism (anthrax) have increased the use of ionizing radiation.

# DISINFECTION

## Physical Methods

### *Filtration*

Membrane filters remove bacteria by mechanical and electrostatic mechanisms

Both live and dead microorganisms can be removed from liquids by positive- or negative-pressure filtration. Membrane filters, usually composed of cellulose esters (eg, cellulose acetate), are available commercially with pore sizes of 0.005 to 1 $\mu$m. For removal of bacteria, a pore size of 0.2 $\mu$m is effective because filters act not only mechanically but by electrostatic adsorption of particles to their surface. Filtration is used for disinfection of large volumes of fluid, especially those containing heat-labile components such as serum. For microorganisms larger than the pore size filtration "sterilizes" these liquids. It is not considered effective for removing viruses.

### *Pasteurization*

Kills vegetative bacteria but not spores

Used for foods and fragile medical equipment

Pasteurization involves exposure of liquids to temperatures in the range 55 to 75°C to remove all vegetative bacteria of significance in human disease. Spores are unaffected by the pasteurization process. Pasteurization is used commercially to render milk safe and extend its storage quality. With the outbreaks of infection due to contamination with enterohemorrhagic *E. coli* (see Chapter 21), this has been extended (reluctantly) to fruit drinks. To the dismay of some of his compatriots, Pasteur proposed application of the process to winemaking to prevent microbial spoilage and vinegarization. Pasteurization in water at 70°C for 30 minutes has been effective and inexpensive when used to render inhalation therapy equipment free of organisms that may otherwise multiply in mucus and humidifying water.

### *Microwaves*

Microwaves kill by generating heat

The use of microwaves in the form of microwave ovens or specially designed units is another method for disinfection. These systems are not under pressure, but they but can achieve temperatures near boiling if moisture is present. In some situations, they are being used as a practical alternative to incineration for disinfection of hospital waste. These procedures cannot be considered sterilization only because the most heat-resistant spores may survive the process.

## Chemical Methods

Most agents are general protoplasmic poisons

Disinfectants are variably inactivated by organic material

Given access and sufficient time, chemical disinfectants cause the death of pathogenic vegetative bacteria. Most of these substances are general protoplasmic poisons and are not currently used in the treatment of infections other than very superficial lesions, having been replaced by antimicrobics (see Chapter 13). Some disinfectants such as the quaternary ammonium compounds, alcohol, and the iodophors reduce the superficial flora and can eliminate contaminating pathogenic bacteria from the skin surface. Other agents such as the phenolics are valuable only for treating inanimate surfaces or for rendering contaminated materials safe. All are bound and inactivated to varying degrees by protein and dirt, and they lose considerable activity when applied to other than clean surfaces. Their activity increases exponentially with increases in temperature, but the relationship between increases in concentration and killing effectiveness is complex and varies for each compound. Optimal in-use concentrations have been established for all available disinfectants. The major groups of compounds currently used are briefly discussed next.

Chemical disinfectants are classified on the basis of their ability to sterilize. High-level disinfectants kill all agents except the most resistant of bacterial spores. Intermediate-level disinfectants kill all agents but not spores. Low-level disinfectants are active against most vegetative bacteria and lipid-enveloped viruses.

Activity against spores and viruses varies

## *Alcohol*

The alcohols are protein denaturants that rapidly kill vegetative bacteria when applied as aqueous solutions in the range 70 to 95% alcohol. They are inactive against bacterial spores and many viruses. Solutions of 100% alcohol dehydrate organisms rapidly but fail to kill, because the lethal process requires water molecules. Ethanol (70–90%) and isopropyl alcohol (90–95%) are widely used as skin decontaminants before simple invasive procedures such as venipuncture. Their effect is not instantaneous, and the traditional alcohol wipe, particularly when followed by a vein-probing finger, is more symbolic than effective, because insufficient time is given for significant killing. Isopropyl alcohol has largely replaced ethanol in hospital use because it is somewhat more active and is not subject to diversion to housestaff parties.

Alcohols require water for maximum effectiveness

Action of alcohol is slow

## *Halogens*

**Iodine** is an effective disinfectant that acts by iodinating or oxidizing essential components of the microbial cell. Its original use was as a tincture of 2% iodine in 50% alcohol, which kills more rapidly and effectively than alcohol alone. This preparation has the disadvantage of sometimes causing hypersensitivity reactions and of staining materials with which it comes into contact. Tincture of iodine has now been largely replaced by preparations in which iodine is combined with carriers (povidone) or nonionic detergents. These agents, termed **iodophors,** gradually release small amounts of iodine. They cause less skin staining and dehydration than tinctures and are widely used in preparation of skin before surgery. Although iodophors are less allergenic than inorganic iodine preparations, they should not be used on patients with a history of iodine sensitivity.

Tincture of iodine in alcohol is effective

Iodophors combine iodine with detergents

**Chlorine** is a highly effective oxidizing agent, which accounts for its lethality to microbes. It exists as hypochlorous acid in aqueous solutions that dissociate to yield free chlorine over a wide pH range, particularly under slightly acidic conditions. In concentrations of less than one part per million, chlorine is lethal within seconds to most vegetative bacteria, and it inactivates most viruses; this efficacy accounts for its use in rendering supplies of drinking water safe and in chlorination of water in swimming pools. Chlorine reacts rapidly with protein and many other organic compounds, and its activity is lost quickly in the presence of organic material. This property, combined with its toxicity, renders it ineffective on body surfaces; however, it is the agent of choice for decontaminating surfaces and glassware that have been contaminated with viruses or spores of pathogenic bacteria. For these purposes it is usually applied as a 5% solution called **hypochlorite.**

Chlorine oxidative action is rapid

Activity is reduced by organic matter

The use of chlorination to disinfect water supplies has proved insufficient in some hospitals because of the relative resistance of *Legionella pneumophila* to the usual concentrations of chlorine. Some institutions have been forced to augment chlorination with systems that add copper and silver ions to the water.

*Legionella* may resist chlorine

## *Hydrogen Peroxide*

Hydrogen peroxide is a powerful oxidizing agent that attacks membrane lipids and other cell components. Although it acts rapidly against many bacteria and viruses, it kills bacteria that produce catalase and spores less rapidly. Hydrogen peroxide has been useful in disinfecting items such as contact lenses that are not susceptible to its corrosive effect.

Hydrogen peroxide oxidizes cell components

## *Surface-Active Compounds*

**Surfactants** are compounds with hydrophobic and hydrophilic groups that attach to and solubilize various compounds or alter their properties. Anionic detergents such as soaps are highly effective cleansers but have little direct antibacterial effect, probably because their charge is similar to that of most microorganisms. Cationic detergents, particularly the **quaternary ammonium compounds** ("quats") such as benzalkonium chloride, are highly bactericidal in the absence of contaminating organic matter. Their hydrophobic and lipophilic groups react with the lipid of the cell membrane of the bacteria, alter the membrane's surface properties and its permeability, and lead to loss of essential cell components and death. These compounds have little toxicity to skin and mucous membranes and, thus, have been used widely for their antibacterial effects in a concentration of 0.1%. They are inactive against spores and most viruses. "Quats" in much higher concentrations than those used in medicine (eg, 5–10%) can be used for sanitizing surfaces.

Hydrophobic and hydrophilic groups of surfactants act on lipids of bacterial membranes

Little activity against viruses

The greatest care is needed in the use of quats because they adsorb to most surfaces with which they come into contact, such as cotton, cork, and even dust. As a result, their concentration may be lowered to a point at which certain bacteria, particularly *Pseudomonas aeruginosa,* can grow in the quat solutions and then cause serious infections. Many instances have been recorded of severe infections resulting from contamination of ophthalmic preparations or of solutions used for treating skin before transcutaneous procedures. It should also be remembered that cationic detergents are totally neutralized by anionic compounds. Thus, the antibacterial effect of quaternary ammonium compounds is inactivated by soap. Because of these problems, quats have been replaced by other antiseptics and disinfectants for most purposes.

"Quats" adsorbed to surfaces may become contaminated with bacteria

Cationic detergents are neutralized by soaps

## *Phenolics*

**Phenol,** one of the first effective disinfectants, was the primary agent employed by Lister in his antiseptic surgical procedure, which preceded the development of aseptic surgery. It is a potent protein denaturant and bactericidal agent. Substitutions in the ring structure of phenol have substantially improved activity and have provided a range of phenols and cresols that are the most effective environmental decontaminants available for use in hospital hygiene. Concern about their release into the environment in hospital waste and sewage has created some pressure to limit their use. This is another of the classic environmental dilemmas of our society: a compound that reduces the risk of disease for one group may raise it for another. Phenolics are less "quenched" by protein than are most other disinfectants, have a detergent-like effect on the cell membrane, and are often formulated with soaps to increase their cleansing property. They are too toxic to skin and tissues to be used as antiseptics, although brief exposures can be tolerated. They are the active ingredient in many mouthwash and sore throat preparations.

Relatively stable to protein

Environmental contamination with phenols and cresols limits use

Two diphenyl compounds, hexachlorophene and chlorhexidine, have been extensively used as skin disinfectants. **Hexachlorophene** is primarily bacteriostatic. Incorporated into a soap, it builds up on the surface of skin epithelial cells over 1 to 2 days of use to produce a steady inhibitory effect on skin flora and Gram-positive contaminants, as long as its use is continued. It was a major factor in controlling outbreaks of severe staphylococcal infections in nurseries during the 1950s and 1960s, but cutaneous absorption was found to produce neurotoxic effects in some premature infants. When it was applied in excessive concentrations, similar problems occurred in older children. It is now a prescription drug.

Skin binding of hexachlorophene enhances effectiveness for staphylococci

Absortion through skin limits use

**Chlorhexidine** has replaced hexachlorophene as a routine hand and skin disinfectant and for other topical applications. It has greater bactericidal activity than hexachlorophene without its toxicity but shares with hexachlorophene the ability to bind to the skin and produce a persistent antibacterial effect. It acts by altering membrane permeability of both Gram-positive and -negative bacteria. It is cationic and, thus, its action is neutralized by soaps and anionic detergents.

Chlorhexidine also binds to skin but is less toxic

## *Glutaraldehyde and Formaldehyde*

Glutaraldehyde and formaldehyde are alkylating agents highly lethal to essentially all microorganisms. Formaldehyde gas is irritative, allergenic, and unpleasant, properties that limit its use as a solution or gas. Glutaraldehyde is an effective high-level disinfecting agent for apparatus that cannot be heat treated, such as some lensed instruments and equipment for respiratory therapy. Formaldehyde vapor, an effective environmental decontaminant under conditions of high humidity, is sometimes used to decontaminate laboratory rooms that have been accidentally and extensively contaminated with pathogenic bacteria, including those such as the anthrax bacillus that form resistant spores. Such rooms are sealed for processing and thoroughly aired before reoccupancy.

Glutaraldehyde is useful for decontamination of equipment

Some risk of infection exists in all health care settings. Hospitalized patients are particularly vulnerable and the hospital environment is complex. The proper matching of the principles and procedures described here to general and specialized situations together with aseptic practices can markedly reduce the risks. The building of such systems is generally referred to as infection control and is discussed further in Chapter 72.

## ADDITIONAL READING

Block SS. *Disinfection, Sterilization, and Preservation.* 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. A standard reference source that contains detailed information.

Widmer AF, Frei R. Decontamination, disinfection, and sterilization. In: Murray PR, ed: *Manual of Clinical Microbiology.* 7th ed. Washington, DC: American Society for Microbiology; 1999. A good account of the practical use of disinfectants, including the meeting of regulatory standards.

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CHAPTER 12

# Epidemiology of Infectious Diseases

W. Lawrence Drew

Epidemiology, the study of the distribution of determinants of disease and injury in human populations, is a discipline that includes both infectious and noninfectious diseases. Most epidemiologic studies of infectious diseases have concentrated on the factors that influence acquisition and spread, because this knowledge is essential for developing methods of prevention and control. Historically, epidemiologic studies and the application of the knowledge gained from them have been central to the control of the great epidemic diseases, such as cholera, plague, smallpox, yellow fever, and typhus.

An understanding of the principles of epidemiology and the spread of disease is essential to all medical personnel, whether their work is with the individual patient or with the community. Most infections must be evaluated in their epidemiologic setting; for example, what infections, especially viral, are currently prevalent in the community? Has the patient recently traveled to an area of special disease prevalence? Is there a possibility of nosocomial infection from recent hospitalization? What is the risk to the patient's family, schoolmates, and work or social contacts?

The recent recognition of emerging infectious diseases has heightened appreciation of the importance of epidemiologic information. A few examples of these newly identified infections are cryptosporidiosis, hantavirus pulmonary syndrome and *Escherichia coli* O157:H7 disease. In addition, some well-known pathogens have assumed new epidemiologic importance by virtue of acquired antimicrobial resistance (eg, penicillin-resistant pneumococci, vancomycin-resistant enterococci, and multiresistant *Mycobacterium tuberculosis*).

Factors that increase the emergence or reemergence of various pathogens include:

- Population movements and the intrusion of humans and domestic animals into new habitats, particularly tropical forests
- Deforestation, with development of new farmlands and exposure of farmers and domestic animals to new arthropods and primary pathogens
- Irrigation, especially primitive irrigation systems, which fail to control arthropods and enteric organisms
- Uncontrolled urbanization, with vector populations breeding in stagnant water
- Increased long-distance air travel, with contact or transport of arthropod vectors and primary pathogens

## SOURCES AND COMMUNICABILITY

Infectious diseases of humans may be caused by exclusively human pathogens, such as *Shigella;* by environmental organisms, such as *Legionella pneumophila;* or by organisms that have their primary reservoir in animals, such as *Salmonella*.

Noncommunicable infections are not spread from person to person but can occur as common-source outbreaks

**Noncommunicable infections** are those that are not transmitted from human to human and include (1) infections derived from the patient's normal flora, such as peritonitis after rupture of the appendix; (2) infections caused by the ingestion of preformed toxins, such as botulism; and (3) infections caused by certain organisms found in the environment, such as clostridial gas gangrene. Some zoonotic infections (diseases transmitted from animals to humans) such as rabies and brucellosis are not transmitted between humans, but others such as plague may be at certain stages. Noncommunicable infections may still occur as common-source outbreaks, such as food poisoning from an enterotoxin-producing *Staphylococcus aureus*–contaminated chicken salad or multiple cases of pneumonia from extensive dissemination of *Legionella* through an air-conditioning system. Because these diseases are not transmissible to others, they do not lead to secondary spread.

Endemic = constant presence

Epidemic = localized outbreak

Pandemic = widespread regional or global epidemic

**Communicable infections** require that an organism be able to leave the body in a form that is directly infectious or is able to become so after development in a suitable environment. The respiratory spread of the influenza virus is an example of direct communicability. In contrast, the malarial parasite requires a developmental cycle in a biting mosquito before it can infect another human. Communicable infections can be **endemic,** which implies that the disease is present at a low but fairly constant level, or **epidemic,** which involves a level of infection above that usually found in a community or population. Communicable infections that are widespread in a region, sometimes worldwide, and have high attack rates are termed **pandemic.**

## INFECTION AND DISEASE

An important consideration in the study of the epidemiology of communicable organisms is the distinction between infection and disease. **Infection** involves multiplication of the organism in or on the host and may not be apparent, for example, during the incubation period or latent when little or no replication is occurring (eg, with herpesviruses). **Disease** represents a clinically apparent response by or injury to the host as a result of infection. With many communicable microorganisms, infection is much more common than disease, and apparently healthy infected individuals play an important role in disease propagation. Inapparent infections are termed **subclinical,** and the individual is sometimes referred to as a **carrier.** The latter term is also applied to situations in which an infectious agent establishes itself as part of a patient's flora or causes low-grade chronic disease after an acute infection. For example, the clinically inapparent presence of *S. aureus* in the anterior nares is termed **carriage,** as is a chronic gallbladder infection with *Salmonella* serotype Typhi that can follow an attack of typhoid fever and result in fecal excretion of the organism for years.

Infection can result in little or no illness

Carriers can be asymptomatic, but infectious to others

With some infectious diseases, such as measles, infection is invariably accompanied by clinical manifestations of the disease itself. These manifestations facilitate epidemiologic control, because the existence and extent of infection in a community are readily apparent. Organisms associated with long incubation periods or high frequencies of subclinical infection such as human immunodeficiency virus (HIV) or hepatitis B virus may propagate and spread in a population for long periods before the extent of the problem is recognized. This makes epidemiologic control more difficult.

## INCUBATION PERIOD AND COMMUNICABILITY

Incubation periods range from a few days to several months

The **incubation period** is the time between exposure to the organism and appearance of the first symptoms of the disease. Generally, organisms that multiply rapidly and produce local infections, such as gonorrhea and influenza, are associated with short incubation periods (eg, 2–4 days). Diseases such as typhoid fever, which depend on hematogenous spread and multiplication of the organism in distant target organs to produce symptoms, often have longer incubation periods (eg, 10 days to 3 weeks). Some diseases have even more prolonged incubation periods because of slow passage of the infecting organism to the target organ, as in rabies, or slow growth of the organism, as in tuberculosis. Incubation periods for one agent may also vary widely depending on route of acquisition and infecting dose; for example, the incubation period of hepatitis B virus infection may vary from a few weeks to several months.

Communicability of a disease in which the organism is shed in secretions may occur primarily during the incubation period. In other infections, the disease course is short but the organisms can be excreted from the host for extended periods. In yet other cases, the symptoms are related to host immune response rather than the organism's action, and thus the disease process may extend far beyond the period in which the etiologic agent can be isolated or spread. Some viruses can integrate into the host genome or survive by replicating very slowly in the presence of an immune response. Such dormancy or latency is exemplified by the herpesviruses, and the organism may emerge long after the original infection and potentially infect others.

Transmission to others can occur before illness onset

The inherent infectivity and virulence of a microorganism are also important determinants of attack rates of disease in a community. In general, organisms of high infectivity spread more easily and those of greater virulence are more likely to cause disease than subclinical infection. The infecting dose of an organism also varies with different organisms and thus influences the chance of infection and development of disease.

## ROUTES OF TRANSMISSION

Various transmissible infections may be acquired from others by direct contact, by aerosol transmission of infectious secretions, or indirectly through contaminated inanimate objects or materials. Some, such as malaria, involve an animate insect vector. These routes of spread are often referred to as **horizontal transmission,** in contrast to **vertical transmission** from mother to fetus. The major horizontal routes of transmission of infectious diseases are summarized in Table 12–1 and discussed next.

Horizontal transmission = direct or indirect person-to-person

Vertical transmission = mother to fetus

### Respiratory Spread

Many infections are transmitted by the respiratory route, often by aerosolization of respiratory secretions with subsequent inhalation by others. The efficiency of this process depends in part on the extent and method of propulsion of discharges from the mouth and nose, the size of the aerosol droplets, and the resistance of the infectious agent to desiccation and inactivation by ultraviolet light. In still air, a particle 100 $\mu$m in diameter requires only seconds to fall the height of a room; a 10-$\mu$m particle remains airborne for about 20 minutes, smaller particles even longer. When inhaled, particles with a diameter of 6 $\mu$m or greater are usually trapped by the mucosa of the nasal turbinates, whereas particles of 0.6 to 5.0 $\mu$m attach to mucous sites at various levels along the upper and lower respiratory tract and may initiate infection. These "droplet nuclei" are most important in transmitting many respiratory pathogens (eg, *M. tuberculosis*). Respiratory secretions are often transferred on hands or inanimate objects (fomites) and may reach the respiratory tract of others in this way. For example, spread of the common cold may involve transfer of infectious secretions from nose to hand by the infected individual, with transfer to others by hand-to-hand contact and then from hand to nose by the unsuspecting victim.

Droplet nuclei are usually less than 6 $\mu$m in size

### Salivary Spread

Some infections, such as herpes simplex and infectious mononucleosis, can be transferred directly by contact with infectious saliva through kissing. Transmission of infectious secretions by direct contact with the nasal mucosa or conjunctiva often accounts for the rapid dissemination of agents such as respiratory syncytial virus and adenovirus. The risk of spread in these instances can be reduced by simple hygienic measures, such as handwashing.

Handwashing is especially important

TABLE 12–1

**Common Routes of Transmission[a]**

| Route of Exit | Route of Transmission | Example |
|---|---|---|
| Respiratory | Aerosol droplet inhalation | Influenza virus; tuberculosis |
| | Nose or mouth → hand or object → nose | Common cold (rhinovirus) |
| Salivary | Direct salivary transfer (eg, kissing) | Oral–labial herpes; infectious mononucleosis, cytomegalovirus |
| | Animal bite | Rabies |
| Gastrointestinal | Stool → hand → mouth and/or stool → object → mouth | Enterovirus infection; hepatitis A |
| | Stool → water or food → mouth | Salmonellosis; shigellosis |
| Skin | Skin discharge → air → respiratory tract | Varicella, poxvirus infection |
| | Skin to skin | Human papilloma virus (warts); syphilis |
| Blood | Transfusion or needle prick | Hepatitis B; cytomegalovirus infection; malaria; AIDS |
| | Insect bite | Malaria; relapsing fever |
| Genital secretions | Urethral or cervical secretions | Gonorrhea; herpes simplex; *Chlamydia* infection |
| | Semen | Cytomegalovirus infection |
| Urine | Urine → hand → catheter | Hospital-acquired urinary tract infection |
| Eye | Conjunctival | Adenovirus |
| Zoonotic | Animal bite | Rabies |
| | Contact with carcasses | Tularemia |
| | Arthropod | Plague; Rocky Mountain spotted fever; Lyme disease |

[a] The examples cited are incomplete, and in some cases more than one route of transmission exists.

## Fecal–Oral Spread

Reduced gastric hydrochloric acid can facilitate enteric infections

Fecal–oral spread involves direct or finger-to-mouth spread, the use of human feces as a fertilizer, or fecal contamination of food or water. Food handlers who are infected with an organism transmissible by this route constitute a special hazard, especially when their personal hygienic practices are inadequate. Some viruses disseminated by the fecal–oral route infect and multiply in cells of the oropharynx and then disseminate to other body sites to cause infection. However, organisms that are spread in this way commonly multiply in the intestinal tract and may cause intestinal infections. They must therefore be able to resist the acid in the stomach, the bile, and the gastric and small-intestinal enzymes. Many bacteria and enveloped viruses are rapidly killed by these conditions, but members of the Enterobacteriaceae and unenveloped viral intestinal pathogens (eg, enteroviruses) are more likely to survive. Even with these organisms, the infecting dose in patients with reduced or absent gastric hydrochloric acid is often much smaller than in those with normal stomach acidity.

## Skin-to-Skin Transfer

Skin-to-skin transfer occurs with a variety of infections in which the skin is the portal of entry, such as the spirochete of syphilis (*Treponema pallidum*), strains of group A streptococci that cause impetigo, and the dermatophyte fungi that cause ringworm and athlete's foot. In most cases, an inapparent break in the epithelium is probably involved in infection. Other diseases may be spread through fomites such as shared towels and inadequately cleansed shower and bath floors. Skin-to-skin transfer usually occurs through abrasions of the epidermis, which may be unnoticed.

Syphilis, ringworm, and impetigo are examples of skin-to-skin transfer

## Bloodborne Transmission

Bloodborne transmission through insect vectors requires a period of multiplication or alteration within an insect vector before the organism can infect another human host. Such is the case with the mosquito and the malarial parasite. Direct transmission from human to human through blood has become increasingly important in modern medicine because of the use of blood transfusions and blood products and the increased self-administration of illicit drugs by intravenous or subcutaneous routes, using shared nonsterile equipment. Hepatitis B and C viruses as well as HIV were frequently transmitted in this way prior to the institution of blood screening tests.

Parenteral drug abuse is a major risk factor

## Genital Transmission

Disease transmission through the genital tract has emerged as one of the most common infectious problems and reflects changing social and sexual mores. Spread can occur between sexual partners or from the mother to the infant at birth. A major factor in these infections has been the persistence, high rates of asymptomatic carriage, and frequency of recurrence of organisms such as *Chlamydia trachomatis*, cytomegalovirus (CMV), herpes simplex virus, and *Neisseria gonorrhoeae*.

Asymptomatic carriage and recurrence are common

## Eye-to-Eye Transmission

Infections of the conjunctiva may occur in epidemic or endemic form. Epidemics of adenovirus and *Haemophilus* conjunctivitis may occur and are highly contagious. The major endemic disease is trachoma, caused by *Chlamydia,* which remains a frequent cause of blindness in developing countries. These diseases may be spread by direct contact via ophthalmologic equipment or by secretions passed manually or through fomites such as towels.

Fomites, unsterile ophthalmologic instruments are associated with transmission

## Zoonotic Transmission

**Zoonotic infections** are those spread from animals, where they have their natural reservoir, to humans. Some zoonotic infections, such as rabies are directly contracted from the bite of the infected animal, while other are transmitted by vectors, especially arthropods (eg, ticks, mosquitoes). Many infections contracted by humans from animals are dead-ended in humans, while others may be transferred between humans once the disease is established in a population. Plague, for example, has a natural reservoir in rodents. Human infections contracted from the bites of rodent fleas may produce pneumonia, which may then spread to other humans by the respiratory droplet route.

Zoonotic = animals to humans

## Vertical Transmission

Certain diseases can spread from the mother to the fetus through the placental barrier. This mode of transmission involves organisms such as rubella virus that can be present in the mother's bloodstream and may occur at different stages of pregnancy with different organisms. Another form of transmission from mother to infant occurs by contact during birth with organisms such as group B streptococci, *C. trachomatis,* and *N. gonorrhoeae,* which colonize the vagina. Herpes simplex virus and CMV can spread by both vertical methods as it may be present in blood or may colonize the cervix. CMV may also be transmitted by breast milk, a third mechanism of vertical transmission.

Vertical transmission can occur transplacentally, during birth, or through breast milk

## EPIDEMICS

Incidence and prevalence rates are usually expressed as number of cases per 100, 1000, or 100,000 population

The characterization of epidemics and their recognition in a community involve several quantitative measures and some specific epidemiologic definitions. **Infectivity,** in epidemiologic terms, equates to attack rate and is measured as the frequency with which an infection is transmitted when there is contact between the agent and a susceptible individual. The **disease index** of an infection can be expressed as the number of persons who develop the disease divided by the total number infected. The **virulence** of an agent can be estimated as the number of fatal or severe cases per the total number of cases. **Incidence,** the number of new cases of a disease within a specified period, is described as a rate in which the number of cases is the numerator and the number of people in the population under surveillance is the denominator. This is usually normalized to reflect a percentage of the population that is affected. **Prevalence,** which can also be described as a rate, is primarily used to indicate the total number of cases existing in a population at risk at a point in time.

Interaction between host and parasite determines extent

The prerequisites for propagation of an epidemic from person to person are a sufficient degree of infectivity to allow the organism to spread, sufficient virulence for an increased incidence of disease to become apparent, and sufficient level of susceptibility in the host population to permit transmission and amplification of the infecting organism. Thus, the extent of an epidemic and its degree of severity are determined by complex interactions between parasite and host. Host factors such as age, genetic predisposition, and immune status can dramatically influence the manifestations of an infectious disease. Together with differences in infecting dose, these factors are largely responsible for the wide spectrum of disease manifestations that may be seen during an epidemic.

Attack rates and disease severity can vary widely by age

The effect of age can be quite dramatic. For example, in an epidemic of measles in an isolated population in 1846, the attack rate for all ages averaged 75%; however, mortality was 90 times higher in children less than 1 year of age (28%) than in those 1 to 40 years of age (0.3%). Conversely, in one outbreak of poliomyelitis, the attack rate of paralytic polio was 4% in children 0 to 4 years of age and 20 to 40% in those 5 to 50 years of age. Sex may be a factor in disease manifestations; for example, the likelihood of becoming a chronic carrier of hepatitis B is twice as high for males as for females.

Immune status of a population influences epidemic behavior

Prior exposure of a population to an organism may alter immune status and the frequency of acquisition, severity of clinical disease, and duration of an epidemic. For example, measles is highly infectious and attacks most susceptible members of an exposed population. However, infection gives solid lifelong immunity. Thus, in unimmunized populations in which the disease is maintained in endemic form, epidemics occur at about 3-year intervals when a sufficient number of nonimmune hosts has been born to permit rapid transmission between them. When a sufficient immune population is reestablished, epidemic spread is blocked and the disease again becomes endemic. When immunity is short-lived or incomplete, epidemics can continue for decades if the mode of transmission is unchecked, which accounts for the present epidemic of gonorrhea.

Immunity in population influences spread

Prolonged and extensive exposure to a pathogen during previous generations selects for a higher degree of innate genetic immunity in a population. For example, extensive exposure of Western urbanized populations to tuberculosis during the 18th and 19th centuries conferred a degree of resistance greater than that among the progeny of rural or geographically isolated populations. The disease spread rapidly and in severe form, for example, when it was first encountered by Native Americans. An even more dramatic example concerns the resistance to the most serious form of malaria that is conferred on peoples of West African descent by the sickle-celled trait (see Chapter 52). These instances are clear cases of natural selection, a process that accounts for many differences in racial immunity.

Sudden appearance of "new" agents can result in pandemic spread

Occasionally, an epidemic arises from an organism against which immunity is essentially absent in a population and that is either of enhanced virulence or appears to be of enhanced virulence because of the lack of immunity. When such an organism is highly infectious, the disease it causes may become pandemic and worldwide. A prime example of this situation is the appearance of a new major antigenic variant of influenza A virus against which there is little if any cross-immunity from recent epidemics with other strains. The 1918–1919 pandemic of influenza was responsible for more deaths than World War I (about 20 million). Subsequent but less serious pandemics have occurred at intervals because of the development of strains of influenza virus with major antigenic shifts (see Chapter 32). Another example, acquired immunodeficiency syndrome (AIDS), illustrates the same principles but also reflects changes in human ecologic and social behavior.

A major feature of serious epidemic diseases is their frequent association with poverty, malnutrition, disaster, and war. The association is multifactorial and includes overcrowding, contaminated food and water, an increase in arthropods that parasitize humans, and the reduced immunity that can accompany severe malnutrition or certain types of chronic stress. Overcrowding and understaffing in day-care centers or institutes for the mentally impaired can similarly be associated with epidemics of infections.

Social and ecological factors determine aspects of epidemic diseases

In recent years, increasing attention has been given to hospital (nosocomial) epidemics of infection. Hospitals are not immune to the epidemic diseases that occur in the community; and outbreaks result from the association of infected patients or persons with those who are unusually susceptible because of chronic disease, immunosuppressive therapy, or the use of bladder, intratracheal, or intravascular catheters and tubes. Control depends on the techniques of medical personnel, hospital hygiene, and effective surveillance. This topic is considered in greater detail in Chapter 72.

Nosocomial = hospital-acquired

## CONTROL OF EPIDEMICS

The first principle of control is recognition of the existence of an epidemic. This recognition is sometimes immediate because of the high incidence of disease, but often the evidence is obtained from ongoing surveillance activities, such as routine disease reports to health departments and records of school and work absenteeism. The causative agent must be identified and studies to determine route of transmission (eg, food poisoning) must be initiated.

Surveillance is the key to recognition of an epidemic

Measures must then be adopted to control the spread and development of further infection. These methods include (1) blocking the route of transmission if possible (eg, improved food hygiene or arthropod control); (2) identifying, treating, and, if necessary, isolating infected individuals and carriers; (3) raising the level of immunity in the uninfected population by immunization; (4) making selective use of chemoprophylaxis for subjects or populations at particular risk of infection, as in epidemics of meningococcal infection; and (5) correcting conditions such as overcrowding or contaminated water supplies that have led to the epidemic or facilitated transfer.

Control measures can vary widely

## GENERAL PRINCIPLES OF IMMUNIZATION

Immunization is the most effective method to provide individual and community protection against many epidemic diseases. Immunization can be active, with stimulation of the body's immune mechanisms through administration of a vaccine, or passive, through administration of plasma or globulin containing preformed antibody to the agent desired. Active immunization with living attenuated organisms generally results in a subclinical or mild illness that duplicates to a limited extent the disease to be prevented. Live vaccines generally provide both local and durable humoral immunity. Killed or subunit vaccines such as influenza vaccine and tetanus toxoid provide immunogenicity without infectivity. They generally involve a larger amount of antigen than live vaccines and must be administered parenterally with two or more spaced injections and subsequent boosters to elicit and maintain a satisfactory antibody level. Immunity usually develops more rapidly with live vaccines, but serious overt disease from the vaccine itself can occur in patients whose immune responses are suppressed. Live attenuated virus vaccines are generally contraindicated in pregnancy because of the risk of infection and damage to developing fetus. Recent developments in molecular biology and protein chemistry have brought greater sophistication to the identification and purification of specific immunizing antigens and epitopes and to the preparation and purification of specific antibodies for passive protection. Thus, immunization is being applied to a broader range of infections.

Prophylaxis or therapy of some infections can be accomplished or aided by passive immunization. This procedure involves administration of preformed antibody obtained from humans, derived from animals actively immunized to the agent, or produced by hybridoma techniques. Animal antisera induce immune responses to their globulins that result in clearance of the passively transferred antibody within about 10 days and carry the risk of hypersensitivity reactions such as serum sickness and anaphylaxis. Human antibodies are less immunogenic and are detectable in the circulation for several weeks after administration. Two types of human antibody preparations are generally available. Immune serum globulin (gamma globulin) is the immunoglobulin G fraction of plasma from a large group of donors that contains antibody to many infectious agents. Hyperimmune globulins are purified antibody preparations from the blood of subjects with high titers of antibody to a specific disease that have resulted from natural exposure or immunization; hepatitis B immune globulin, rabies immune globulin, and human tetanus immune globulin are examples. Details of the use of these globulins can be obtained from the chapters that discuss the diseases in question. Passive antibody is most effective when given early in the incubation period.

Passive immunization has a temporary effect

## ADDITIONAL READING

American Academy of Pediatrics. Report of the Committee on Infectious Diseases. *Red Book 2000,* 25th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2000. This manual is published every 3 years, with periodic interim updates as necessary. A comprehensive resource on immunization recommendations, it contains much other information regarding the epidemiology and control of infectious diseases.

Gladwell M. The Dead Zone. *The New Yorker* 1997; Sept 29:52–56. A fascinating lay person's account of an attempt to define the cause of the influenza pandemic of 1918.

Ryan ET, Kain KC. Health advice and immunization for travelers. *N Engl J Med* 2000;342:1716–1725. This review includes consideration of noninfectious as well as infectious risks for travelers and ways to minimize them.

US Department of Health and Human Services. Health Information for International Travel, 2001–2002. www.cdc.gov/travel/yb/index.htm. This reference is the most timely and up-to-date source for travel recommendations, including updates on infectious outbreaks around the world.

US Public Health Service. Impact of vaccines universally recommended for children, 1990–1998. *MMWR* 2000;48:243–248. This report summarizes the dramatic effects on infectious diseases by routine immunization in the last decade of the 20th century.

CHAPTER 13

# Antibacterial and Antiviral Agents

KENNETH J. RYAN AND W. LAWRENCE DREW

The ability to direct therapy specifically at a disease-causing infectious agent is unique to the management of infectious diseases. Its initial success depends on exploiting differences between our own makeup and metabolism and that of the microorganism in question. The mode of action of antimicrobials on bacteria and viruses is the focus of this chapter. The continued success of antibacterial and antiviral agents depends on whether the organisms to which the agent was originally directed develop resistance. Resistance to antibacterial agents is the subject of Chapter 14. Specific information about pathogenic organisms can be found in later chapters; a complete guide to the treatment of infectious diseases is beyond the scope of this book.

Natural materials with some activity against microbes were used in folk medicine in earlier times, such as the bark of the cinchona tree (containing quinine) in the treatment of malaria. Rational approaches to chemotherapy began with Ehrlich's development of arsenical compounds for the treatment of syphilis early in the 20th century. Many years then elapsed before the next major development, which was the discovery of the therapeutic effectiveness of a sulfonamide (prontosil rubrum) by Domagk in 1935. Penicillin, which had been discovered in 1929 by Fleming, could not be adequately purified at that time; however, this was accomplished later, and penicillin was produced in sufficient quantities so that Florey and his colleagues could demonstrate its clinical effectiveness in the early 1940s. The first antiviral agent, methisazone, was a derivative of sulfonamides shown to be active against pox viruses. Numerous new antimicrobial agents have been discovered or developed, and many have found their way into clinical practice.

Sulfonamides and penicillin were the first effective antibacterial agents

## ANTIBACTERIAL THERAPY

### GENERAL CONSIDERATIONS

Clinically effective antimicrobial agents all exhibit selective toxicity toward the parasite rather than the host, a characteristic that differentiates them from the disinfectants (see Chapter 11). In most cases, selective toxicity is explained by action on microbial processes or structures that differ from those of mammalian cells. For example, some agents act on bacterial cell wall synthesis, and others on functions of the 70 S bacterial

Ideally, selective toxicity is based on the ability of an antimicrobial agent to attack a target present in bacteria but not humans

ribosome but not the 80 S eukaryotic ribosome. Some antimicrobial agents, such as penicillin, are essentially nontoxic to the host, unless hypersensitivity has developed. For others, such as the aminoglycosides, the effective therapeutic dose is relatively close to the toxic dose; as a result, control of dosage and blood level must be much more precise.

## Definitions

- **Antibiotic**—antimicrobials of microbial origin, most of which are produced by fungi or by bacteria of the genus *Streptomyces.*
- **Antimicrobial, antimicrobic**—any substance with sufficient antimicrobial activity that it can be used in the treatment of infectious diseases.
- **Bactericidal**—an antimicrobial that not only inhibits growth but is lethal to bacteria.
- **Bacteriostatic**—an antimicrobial that inhibits growth but does not kill the organisms.
- **Chemotherapeutic**—a broad term that encompasses antibiotics, antimicrobials, and drugs used in the treatment of cancer. In the context of infectious diseases, it implies the agent is not an antibiotic.
- **Minimal inhibitory concentration (MIC)**—a laboratory term that defines the lowest concentration ($\mu$g/mL) able to inhibit growth of the microorganism.
- **Resistant**—organisms that are not inhibited by clinically achievable concentrations of a antimicrobial agent.
- **Sensitive**—term applied to microorganisms indicating that they will be inhibited by concentrations of the antimicrobic that can be achieved clinically.
- **Spectrum**—an expression of the categories of microorganisms against which an antimicrobial is typically active. A narrow-spectrum agent has activity against only a few organisms. A broad-spectrum agent has activity against organisms of diverse types (eg, Gram-positive and Gram-negative bacteria).
- **Susceptible**—term applied to microorganisms indicating that they will be inhibited by concentrations of the antimicrobic that can be achieved clinically.

## Sources of Antimicrobial Agents

Antibiotics are synthesized by molds or bacteria

Production in quantity is by industrial fermentation

There are several sources of antimicrobial agents. The antibiotics are of biological origin and probably play an important part in microbial ecology in the natural environment. Penicillin, for example, is produced by several molds of the genus *Penicillium,* and the prototype cephalosporin antibiotics were derived from other molds. The largest source of naturally occurring antibiotics is the genus *Streptomyces,* the members of which are Gram-positive, branching bacteria found in soils and freshwater sediments. Streptomycin, the tetracyclines, chloramphenicol, erythromycin, and many other antibiotics were discovered by screening large numbers of *Streptomyces* isolates from different parts of the world. Antibiotics are mass produced by techniques derived from the procedures of the fermentation industry.

Chemicals with antibacterial activity are discovered by chance or as the result of screening programs

Chemically synthesized antimicrobial agents were initially discovered among compounds synthesized for other purposes and tested for their therapeutic effectiveness in animals. The sulfonamides, for example, were discovered as a result of routine screening of aniline dyes. More recently, active compounds have been synthesized with structures tailored to be effective inhibitors or competitors of known metabolic pathways. Trimethoprim, which inhibits dihydrofolate reductase, is an excellent example.

Naturally occurring antimicrobics can be chemically modified

A third source of antimicrobial agents is molecular manipulation of previously discovered antibiotics or chemotherapeutics to broaden their range and degree of activity against microorganisms or to improve their pharmacologic characteristics. Examples include the development of penicillinase-resistant and broad-spectrum penicillins, as well as a large range of aminoglycosides and cephalosporins of increasing activity, spectrum, and resistance to inactivating enzymes.

## Spectrum of Action

Spectrum is the range of bacteria against which the agent is typically active

The **spectrum** of activity of each antimicrobic describes the genera and species against which it is typically active. For the most common antimicrobics and bacteria, these are shown in Table 13-1. Spectra overlap but are usually characteristic for each broad class of

TABLE 13–1

## Usual Susceptibility Patterns of Common Bacteria to Some Commonly Used Bacteriostatic and Bactericidal Antimicrobial Agents

| Antimicrobic | Bactericidal | Bacteriostatic | *Staphylococcus aureus* | *Enterococci* | Other *Streptococci* | *Neisseria* | *Haemophilus* | *Legionella* | *Mycoplasma* | *Escherichia coli* | *Proteus mirabilis* | Other *Proteus spp* | *Klebsiella* | *Enterobacter* | *Serratia* | *Pseudomonas aeruginosa* | *Bacteroides fragilis* | Other Gram-negative Anaerobes | *Clostridium* | *Rickettsia* | *Chlamydia* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Benzyl penicillin | + | | 1 ◕ | C ◍◔ | 1 ◔ | 1 ◔ | ◍◔ | ● | ● | ● | ◍ | ● | ● | ● | ● | ● | ● | 1 ◔ | 1 ○ | ● | ● | Narrow-spectrum agents |
| Penicillinase-resistant penicillins | + | | 1 ◔ | ● | 2 ◔ | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | |
| Erythromycin | ± | + | 2 ◔ | 2 ◔ | 2 ◔ | ◔ | | 1 ○ | 1 ○ | ● | ● | ● | ● | ● | ● | ● | – | – | – | – | 2 ○ | |
| Clindamycin | ± | + | 2 ◔ | – | ◔ | ● | ● | – | – | ● | ● | ● | ● | ● | ● | ● | ◔ | ◔ | ◔ | – | – | |
| Vancomycin | + | | 2 ◔ | 1 ◔ | 2 ○ | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | – | – | 1 ○ | – | – | |
| Ampicillin | + | | 2 ◕ | 1 ◔ | 2 ◔ | 1 ◔ | 1 ◔ | ● | ● | 1 ◔ | 1 ◔ | ● | ● | ● | ● | ● | ● | – | 1 ○ | ● | ● | Broad-spectrum agents |
| Piperacillin | + | | – | ○ | ○ | – | ◔ | ● | ● | – | 1 ○ | 1 ◔ | 1 ◔ | 1 ◔ | 1 ◔ | 1 ◔ | 2 ◔ | 1 ○ | – | ● | ● | |
| Cephalothin | + | | 2 ◔ | ● | 2 ◔ | – | ● | ● | ● | ◔ | ○ | ● | ◔ | ● | ● | ● | ● | ◔ | ◔ | ● | ● | |
| Cefotetan | + | | – | ● | 1 ○ | 1 ○ | – | ● | ● | 1 ○ | 1 ○ | 1 ○ | 1 ○ | ● | ◔ | ● | 2 ◔ | ○ | – | ● | ● | |
| Ceftazidime | + | | – | – | – | – | ○ | ● | ● | 1 ○ | 1 ○ | 1 ○ | 1 ○ | 2 ◔ | 2 ◔ | ◔ | ◔ | – | – | ● | ● | |
| Imipenem | + | | 2 ○ | 2 ○ | 2 ○ | 1 ○ | 1 ○ | – | ● | 1 ○ | 1 ○ | 1 ○ | 1 ○ | 1 ○ | 1 ○ | 1 ◔ | 1 ○ | 1 ○ | 1 ○ | – | – | |
| Aztreonam | + | | ● | ● | ● | 1 ○ | 1 ○ | – | ● | 1 ◔ | 1 ◔ | 1 ○ | 1 ○ | ◔ | 1 ◔ | 1 ◔ | ● | ● | ● | – | – | |
| Gentamicin | + | | ◔ | C ● | ● | – | – | ● | – | 1 ◔ | 1 ○ | 1 ○ | 1 ◔ | 1 ◔ | 1 ◔ | 1 ◔ | ● | ● | ● | – | – | |
| Amikacin | + | | ◔ | C ● | ● | – | – | ● | – | 1 ◔ | 1 ○ | 1 ○ | 1 ◔ | 1 ◔ | 1 ◔ | 1 ◔ | ● | ● | ● | – | – | |
| Tetracycline | | + | ◔ | ● | ◕ | ◔ | ○ | 2 ○ | 1 ○ | ◔ | ● | ● | ◔ | ◔ | ◕ | ● | ◕ | ◔ | ◔ | 1 ○ | 1 ○ | |
| Chloramphenicol | | + | ◔ | ◔ | ◔ | ◔ | ◔ | 2 ● | – | ◔ | ◔ | ◔ | ◔ | ◔ | ◕ | ● | 1 ◔ | 2 ○ | – | 1 ○ | – | |
| Ciprofloxacin | + | | ◔ | ◍ | ◍ | 2 ○ | – | – | ● | 1 ◔ | 1 ○ | 1 ○ | 1 ○ | 1 ○ | 1 ○ | 2 ◔ | ● | ● | – | – | ◍ | |
| Sulfamethoxazole + trimethoprim | ± | + | – | – | – | – | 1 ◔ | – | – | 1 ◔ | ◔ | ◔ | ◔ | ◔ | ◔ | ● | ◔ | – | – | – | 3 – | |

Proportions of susceptible and resistant strains: ○ , 100% susceptible ◔ , 25% resistant ● , 100% resistant ◍ , intermediate susceptibility.

Abbreviations: – = no present indication for therapy or insufficient data 1 = antimicrobic of choice for susceptible strains 2 = second-line agent
3 = *c. trachomatis*-sensitive, *c. psittaci*-resistant C = useful in combinations of a ß-lactam and an aminoglycoside

antimicrobic. Some antibacterial antimicrobics are known as **narrow-spectrum agents;** for example, benzyl penicillin is highly active against many Gram-positive and Gram-negative cocci but has little activity against enteric Gram-negative bacilli. Chloramphenicol, tetracycline, and the cephalosporins, on the other hand, are **broad-spectrum agents** that inhibit a wide range of Gram-positive and Gram-negative bacteria, including some obligate

Broad-spectrum agents inhibit both Gram-positive and Gram-negative species intracellular organisms. When resistance develops in an initially sensitive genus or species, that species is still considered within the spectrum even when the resistant subpopulation is significant. For example, the spectrum of benzyl penicillin is considered to include *Staphylococcus aureus,* although more than 80% of strains now are penicillin resistant.

## SELECTED ANTIBACTERIAL ANTIMICROBICS

Various aspects of the major antimicrobics are now considered in more detail, with emphasis on their modes of action and spectrum. Resistance is mentioned here in the context of spectrum, with mechanisms of resistance covered in Chapter 14. Details on specific antimicrobic use, dosage, and toxicity should be sought in one of the specialized texts or handbooks written for that purpose.

### Antimicrobics That Act on Cell Wall Synthesis

Cross-linking of peptidoglycan is the target of β-lactams and glycopeptides

The peptidoglycan (murein sac) component of the bacterial cell wall gives it its shape and rigidity. This giant molecule is formed by weaving the linear glycans *N*-acetylglucosamine and *N*-acetylmuramic acid into a basket-like structure. Mature peptidoglycan is held together by cross-linking of short peptide side chains hanging off the long glycan molecules. This cross-linking process is the target of two of the most important groups of antimicrobics, the β-lactams and the glycopeptides (vancomycin and teicoplanin) (Fig 13–1). Peptidoglycan is unique to bacteria and its synthesis is described in more detail in Chapter 2.

### *β-Lactam Antimicrobics*

A β-lactam ring is part of the structure of all β-lactam antimicrobics

The β-lactam antimicrobics comprise the penicillins, cephalosporins, carbapenems, and monobactams. Their name derives from the presence of a β-lactam ring in their structure; this ring is essential for antibacterial activity. Penicillin, the first member of this class, was derived from molds of the genus *Penicillium,* and later natural β-lactams were derived from both molds and bacteria of the genus *Streptomyces.* Today it is possible to synthesize β-lactams, but most are derived from semisynthetic processes involving the chemical modification of the products of fermentation.

Interfere with peptidoglycan cross-linking by binding to transpeptidases called PBPs

The β-lactam antimicrobics interfere with the transpeptidation reactions that seal the peptide crosslinks between glycan chains. They do so by interference with the action of the transpeptidase enzymes which carry out this cross-linking. These targets of all the β-lactams are commonly called penicillin-binding proteins (PBPs), reflecting the stereochemical nature of their interference, which was first described in experiments with penicillin. Several distinct PBPs occur in any one strain, are usually species specific, and vary in the avidity of their binding to different β-lactam antimicrobics.

Penicillins, cephalosporins, monobactams, and carbapenems differ in terms of the structures fused to the β-lactam ring

The β-lactams are classified by chemical structure (Fig 13–2). They may have one β-lactam ring (monobactams), or a β-lactam ring fused to a five-member penem ring (penicillins, carbapenems), or a six-member cephem ring (cephalosporins). Within these major groups, differences in the side chain(s) attached to the single or double ring can have a significant effect on the pharmacologic properties and spectrum of any β-lactam. The pharmacologic properties include resistance to gastric acid, which allows oral administration, and their pattern of distribution into body compartments (eg, blood, cerebrospinal fluid, joints). The features that alter the spectrum include permeability into the bacterial cell, affinity for PBPs, and vulnerability to the various bacterial mechanisms of resistance.

β-Lactam antimicrobics kill growing bacteria killed by lysing weakened cell walls

β-Lactam antimicrobics are usually highly bactericidal, but only to growing bacteria synthesizing new cell walls. Killing involves attenuation and disruption of the developing peptidoglycan "corset," liberation or activation of autolytic enzymes that further disrupt weakened areas of the wall, and finally osmotic lysis from passage of water through the cytoplasmic membrane to the hypertonic interior of the cell. As might be anticipated, cell wall–deficient organisms, such as *Mycoplasma,* are not susceptible to β-lactam antimicrobics.

***Penicillins*** Penicillins differ primarily in their spectrum of activity against Gram-negative bacteria and resistance to staphylococcal penicillinase. This penicillinase is one

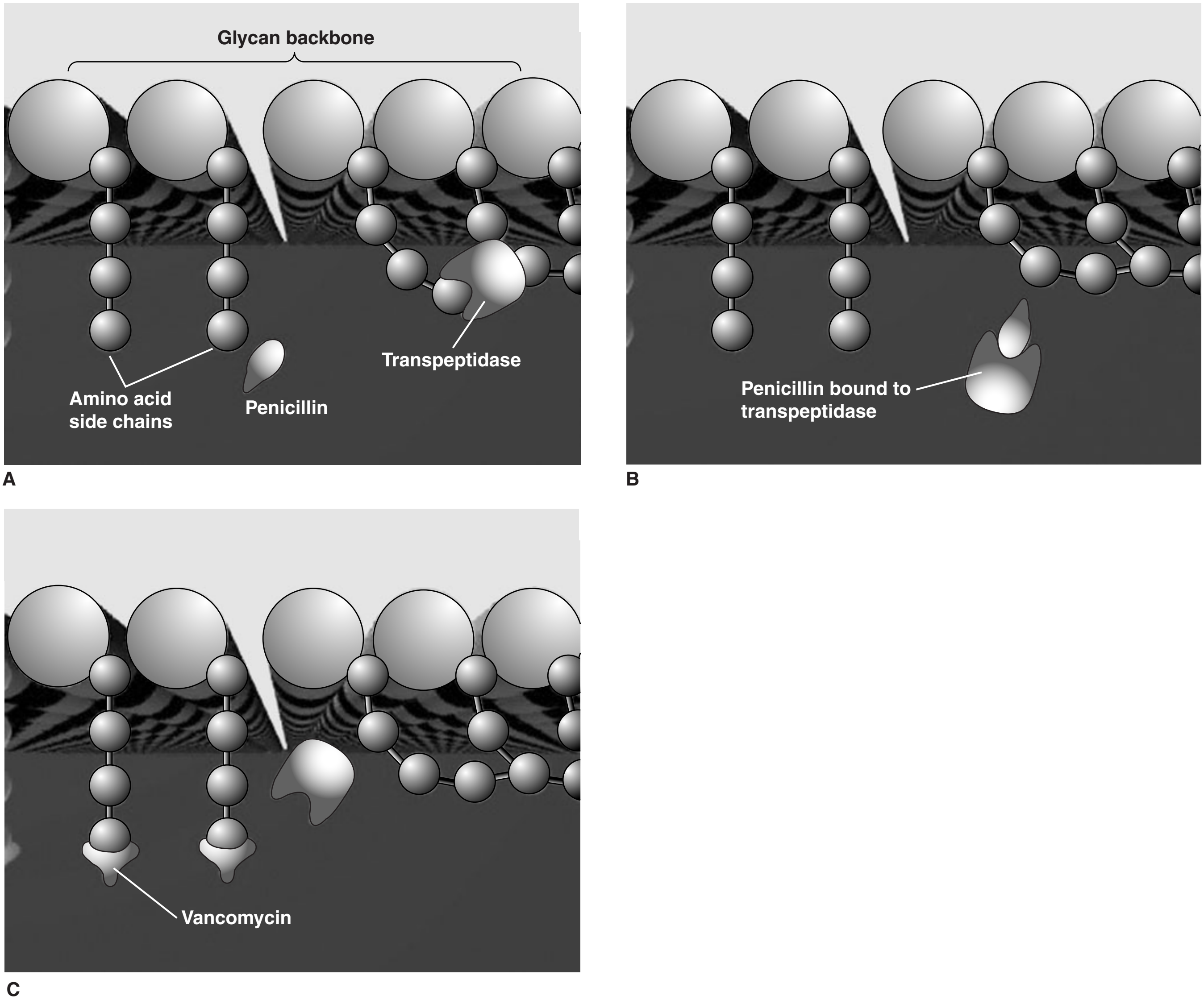


**FIGURE 13–1**
Action of antimicrobics on peptidoglycan synthesis. The glucan backbone and the amino acid side chains of peptidoglycan are shown. The transpeptidase enzyme catalyzes the cross-linking of the amino acid side chains. Penicillin and other $\beta$-lactams bind to the transpeptidase, preventing it from carrying out its function. Vancomycin binds directly to the amino acids, preventing the binding of transpeptidase.

of a family of bacterial enzymes called $\beta$-lactamases that inactivate $\beta$-lactam antimicrobics (see Chapter 14). **Penicillin G** is active primarily against Gram-positive organisms, Gram-negative cocci, and some spirochetes, including the spirochete of syphilis. They have little action against most Gram-negative bacilli, because the outer membrane prevents passage of these antibiotics to their sites of action on cell wall synthesis. Penicillin G is the least toxic and least expensive of all the penicillins. Its modification as penicillin V confers acid stability, so it can be given orally.

Resistance to staphylococcal and Gram-negative $\beta$-lactamases determines spectrum

Penetration of outer membrane is often limited

The penicillinase-resistant penicillins **(methicillin, nafcillin, oxacillin)** also have narrow spectra, but are active against penicillinase-producing *S. aureus*. The broader spectrum penicillins owe their expanded activity to the ability to traverse the outer membrane of some Gram-negative bacteria. Some, such as ampicillin, have excellent activity against a range of Gram-negative pathogens but not *P. aeruginosa,* an important opportunistic pathogen.

Broad-spectrum penicillins penetrate the outer membrane of some Gram-negative bacteria

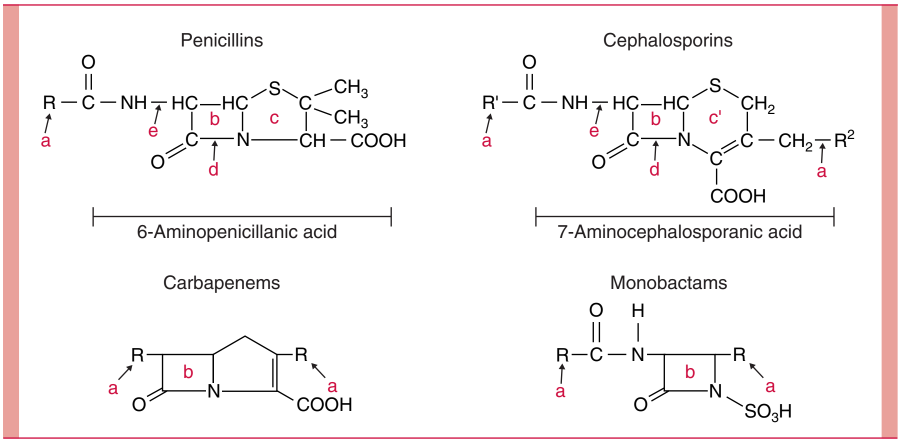


**FIGURE 13–2** Basic structure of $\beta$-lactam antibiotics. a, Different side chains determine degree of activity, spectrum, pharmacologic properties, resistance to $\beta$-lactamases; b, $\beta$-lactam ring; c, thiazolidine ring; c′ dihydrothiazine ring; d, site of action of $\beta$-lactamases; e, site of action of amidase.

Some penicillins are inactivated by staphylococcal penicillinase

Others, such as **carbenicillin** and **ticarcillin,** are active against *Pseudomonas* when given in high dosage but are less active than ampicillin against some other Gram-negative organisms. The penicillins with a Gram-negative spectrum are slightly less active than penicillin G against Gram-positive organisms and are inactivated by staphylococcal penicillinase.

Cephalosporins are penicillinase resistant

Shifting between first- and third-generation cephalosporins gives a wider Gram-negative spectrum

Second- and third-generation cephalosporins have less activity against Gram-positive bacteria

***Cephalosporins*** The structure of the cephalosporins confers resistance to hydrolysis by staphylococcal penicillinase and to the $\beta$-lactamases of groups of Gram-negative bacilli, which vary with each cephalosporin. The cephalosporins are classified by generation—first, second, third, or fourth. The "generation" term relates to historical breakthroughs in expanding their spectrum through modification of the side chains. In general, a cephalosporin of a higher generation has a wider spectrum, in some instances, more quantitative activity (lower minimum inhibitory concentration; MIC) against Gram-negative bacteria. As the Gram-negative spectrum increases, these agents typically lose some of their potency (higher MIC) against Gram-positive bacteria.

First-generation cephalosporins inhibit Gram-positive bacteria and a few Enterobacteriaceae

The first-generation cephalosporins **cefazolin** and **cephalexin** have a spectrum of activity against Gram-positive organisms that resembles that of the penicillinase-resistant penicillins, and in addition, they are active against some of the Enterobacteriaceae (see Table 13–1). These agents continue to have therapeutic value because of their high activity against Gram-positive organisms and because a broader spectrum may be unnecessary.

Second-generation cephalosporins are also active against anaerobes

Second-generation cephalosporins **cefoxitin** and **cefaclor** are resistant to $\beta$-lactamases of some Gram-negative organisms that inactivate first-generation compounds. Of particular importance is their expanded activity against Enterobacteriaceae species and against anaerobes such as *Bacteroides fragilis.*

Third-generation cephalosporins have increasing potency against Gram-negative organisms

Ceftriaxone and cefotaxime are preferred for meningitis

Ceftazidime is used for *Pseudomonas*

Third-generation cephalosporins, such as **ceftriaxone, cefotaxime,** and **ceftazidime,** have an even wider spectrum; they are active against Gram-negative organisms, often at MICs that are 10- to 100-fold lower than first-generation compounds. Of these three agents, only ceftazidime is consistently active against *P. aeruginosa.* The potency, broad spectrum, and low toxicity of the third-generation cephalosporins have made them the preferred agents in life-threatening infections in which the causative organism has not yet been isolated. Selection depends on the clinical circumstances. For example, ceftriaxone or cefotaxime is preferred for childhood meningitis because it has the highest activity against the three major causes, *Neisseria meningitidis, Streptococcus pneumoniae,* and *Haemophilus influenzae.* For a febrile bone marrow transplant patient, ceftazidime might be chosen because of the prospect of *P. aeruginosa* involvement.

Fourth-generation cephalosporins have enhanced ability to penetrate outer membrane

Fourth-generation cephalosporins have enhanced ability to cross the outer membrane of Gram-negative bacteria as well as resistance to many Gram-negative $\beta$-lactamases. Compounds such as **cefepime** have activity against an even wider spectrum of Enterobacteriaceae as well as *P. aeruginosa*. These cephalosporins retain the high affinity of third-generation drugs and activity against *Neisseria* and *H. influenzae*.

***Carbapenems*** The carbapenems **imipenem** and **meropenem** have the broadest spectrum of all $\beta$-lactam antibiotics. This fact appears to be due to the combination of easy penetration of Gram-negative and Gram-positive bacterial cells and high level of resistance to β-lactamases. Both agents are active against streptococci, more active than cephalosporins against staphylococci, and highly active against both β-lactamase-positive and -negative strains of gonococci and *H. influenzae.* In addition, they are as active as third-generation cephalosporins against Gram-negative rods, and effective against obligate anaerobes. Imipenem is rapidly hydrolyzed by a renal tubular dehydropeptidase-1; therefore, it is administered together with an inhibitor of this enzyme (cilastatin), which greatly improves its urine levels and other pharmacokinetic characteristics. Meropenem is not significantly degraded by dehydropeptidase-1 and does not require coadministration of cilastatin.

Carbapenems are very broad spectrum

***Monobactams*** **Aztreonam,** the first monobactam licensed in the United States, has a spectrum limited to aerobic and facultatively anaerobic Gram-negative bacteria, including Enterobacteriaceae, *P. aeruginosa, Haemophilus,* and *Neisseria*. Monobactams have poor affinity for the PBPs of Gram-positive organisms and anaerobes and thus little activity against them, but they are highly resistant to hydrolysis by β-lactamases of Gram-negative bacilli. Anaerobic superinfections and major distortions of the bowel flora are less common with aztreonam therapy than with other broad-spectrum β-lactam antimicrobics, presumably because aztreonam does not produce a general suppression of gut anaerobes.

Activity is primarily against Gram-negatives

***β-Lactamase Inhibitors*** A number of β-lactams with little or no antimicrobic activity are capable of binding irreversibly to β-lactamase enzymes and, in the process, rendering them inactive. Three such compounds, **clavulanic acid, sulbactam,** and **tazobactam,** are referred to as suicide inhibitors, because they must first be hydrolyzed by a β-lactamase before becoming effective inactivators of the enzyme. They are highly effective against staphylococcal penicillinases and broad-spectrum β-lactamases; however, their ability to inhibit cephalosporinases is significantly less. Combinations of one of these inhibitors with an appropriate β-lactam antimicrobic protects the therapeutic agent from destruction by many β-lactamases and significantly enhances its spectrum. Four such combinations are now available in the United States: amoxicillin/clavulanate, ticarcillin/clavulanate, ampicillin/sulbactam, and piperacillin/tazobactam. Bacteria that produce chromosomally encoded inducible cephalosporinases are not susceptible to these combinations. Whether these combinations offer therapeutic or economic advantages compared with the β-lactamase–stable antibiotics now available remains to be determined.

β-lactamase inhibitors are β-lactams that bind β-lactamases

Other β-lactams are enhanced in the presence of β-lactamase inhibitors

***Clinical Use*** The β-lactam antibiotics are usually the drugs of choice for infections by susceptible organisms because of their low toxicity and bactericidal action. They have also proved of great value in the prophylaxis of many infections. They are excreted by the kidney and achieve very high urinary levels. Penicillins reach the cerebrospinal fluid when the meninges are inflamed and are effective in the treatment of meningitis, but first- and second-generation cephalosporins are not. In contrast, the third-generation cephalosporins penetrate much better and have become the agents of choice in the treatment of undiagnosed meningitis and meningitis caused by most Gram-negative organisms.

Low toxicity favors use of all β-lactams

## *Glycopeptide Antimicrobics*

Two agents, **vancomycin** and **teicoplanin,** belong to this group. Each of these antimicrobics inhibit assembly of the linear peptidoglycan molecule by binding directly to the terminal amino acids of the peptide side chains. The effect is the same as with β-lactams, prevention of peptidoglycan cross-linking. Both agents are bactericidal, but are primarily active only against Gram-positive bacteria. Their main use has been against multiresistant Gram-positive infections including those caused by strains of staphylococci that are resistant to the penicillinase-resistant penicillins and cephalosporins. Neither agent is absorbed by mouth, although both have been used orally to treat *Clostridium difficile* infections of the bowel (see Chapter 19).

Glycopeptide antimicrobics bind directly to amino acid side chains

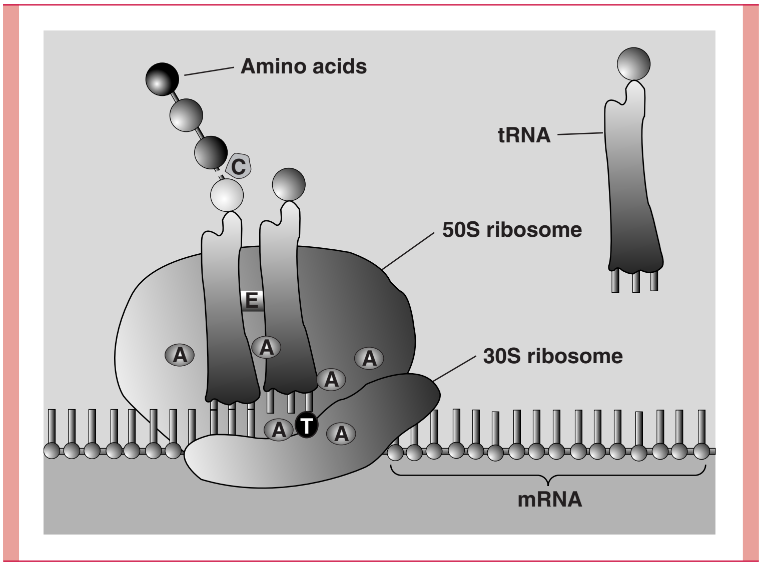


**FIGURE 13–3**
Action of antimicrobics on protein synthesis. Aminoglycosides (A) bind to multiple sites on both the 30S and 50S ribosomes in a manner that prevents the tRNA from forming initiation complexes. Tetracyclines (T) act in a similar manner, binding only to the 30S ribosomes. Chloramphenicol (C) blocks formation of the peptide bond between the amino acids. Erythromycin (E) and macrolides block the translocation of tRNA from the acceptor to the donor side on the ribosome.

## Inhibitors of Protein Synthesis (Fig 13–3)

### *Aminoglycosides*

Aminoglycosides must be transported into cell by oxidative metabolism

Not active against anaerobes

All members of the aminoglycoside group of antimicrobics have a six-member aminocyclitol ring with attached amino sugars. The individual agents differ in terms of the exact ring structure and the number and nature of the amino sugar residues. Aminoglycosides are active against a wide range of bacteria, but only those organisms that are able to transport them into the cell by a mechanism that involves oxidative phosphorylation. Thus, they have little or no activity against strict anaerobes or facultative organisms that metabolize only fermentatively (eg, streptococci). It appears highly probable that aminoglycoside activity against facultative organisms is similarly reduced in vivo when the oxidation–reduction potential is low.

Ribosome binding disrupts initiation complexes

Newer agents bind to multiple sites

Once inside bacterial cells, aminoglycosides inhibit protein synthesis by binding to the bacterial ribosomes either directly or by involving other proteins. This binding destabilizes the ribosomes, blocks initiation complexes, and thus prevents elongation of polypeptide chains. The agents may also cause distortion of the site of attachment of mRNA, mistranslation of codons, and failure to produce the correct amino acid sequence in proteins. The first aminoglycoside, streptomycin, is bound to the 30S ribosomal subunit, but the newer and more active aminoglycosides bind to multiple sites on both 30S and 50S subunits. This gives the newer agents broader spectrum and less susceptibility to resistance due to binding site mutation.

No entry into human cells

Eukaryotic ribosomes are resistant to aminoglycosides, and the antimicrobics are not actively transported into eukaryotic cells. These properties account for their selective toxicity and also explain their ineffectiveness against intracellular bacteria such as *Rickettsia* and *Chlamydia*.

Spectum includes *P. aeruginosa*

**Gentamicin** and **tobramycin** are the major aminoglycosides; they have an extended spectrum, which includes staphylococci; Enterobacteriaceae; and of particular importance, *P. aeruginosa.* **Streptomycin** and **amikacin** are now primarily used in combination with other antimicrobics in the therapy of tuberculosis and other mycobacterial diseases. **Neomycin,** the most toxic aminoglycoside, is used in topical preparations and as an oral preparation before certain types of intestinal surgery, because it is poorly absorbed.

Renal and vestibular toxicity must be monitored

All of the aminoglycosides are toxic to the vestibular and auditory branches of the eighth cranial nerve to varying degrees; this damage can lead to complete and irreversible loss of hearing and balance. These agents may also be toxic to the kidneys. It is often essential to monitor blood levels during therapy to ensure adequate yet nontoxic doses, especially when renal impairment diminishes excretion of the drug. For example, blood levels of gentamicin should be below 10 $\mu$g/mL to avoid nephrotoxicity, but many strains of *P. aeruginosa* require 2 to 4 $\mu$g/mL for inhibition.

The clinical value of the aminoglycosides is a consequence of their rapid bactericidal effect, their broad spectrum, the slow development of resistance to the agents now most often used, and their action against *Pseudomonas* strains that resist many other antimicrobics. They cause fewer disturbances of the normal flora than most other broad-spectrum antimicrobics, probably because of their lack of activity against the predominantly anaerobic flora of the bowel, and because they are only used parenterally for systemic infections. The $\beta$-lactam antibiotics often act synergistically with the aminoglycosides, most likely because their action on the cell wall facilitates aminoglycoside penetration into the bacterial cell. This effect is most pronounced with organisms such as streptococci and enterococci, which lack the metabolic pathways required to transport aminoglycosides to their interior.

Broad spectrum and slow development of resistance enhance use

Often combined with $\beta$-lactam antimicrobics

## *Tetracyclines*

Tetracyclines are composed of four fused benzene rings. Substitutions on these rings provide differences in pharmacologic features of the major members of the group, **tetracycline, minocycline,** and **doxycycline.** The tetracyclines inhibit protein synthesis by binding to the 30S ribosomal subunit at a point that blocks attachment of aminoacyl-tRNA to the acceptor site on the mRNA ribosome complex. Unlike the aminoglycosides, their effect is reversible; they are bacteriostatic rather than bactericidal.

Tetracyclines block tRNA attachment

Activity is bacteriostatic

The tetracyclines are broad-spectrum agents with a range of activity that encompasses most common pathogenic species, including Gram-positive and Gram-negative rods and cocci and both aerobes and anaerobes. They are active against cell wall–deficient organisms, such as *Mycoplasma* and spheroplasts, and against some obligate intracellular bacteria, including members of the genera *Rickettsia* and *Chlamydia*. Differences in spectrum of activity between members of the group are relatively minor. Acquired resistance to one generally confers resistance to all.

Broad spectrum includes some intracellular bacteria

The tetracyclines are absorbed orally. In practice, they are divided into those agents that generate blood levels for only a few hours and those that are longer-acting (minocycline and doxycycline), which can be administered less often. The tetracyclines are chelated by divalent cations, and their absorption and activity are reduced. Thus, they should not be taken with dairy products or many antacid preparations. Tetracyclines are excreted in the bile and urine in active form.

Orally absorbed but chelated by some foods

The tetracyclines have a strong affinity for developing bone and teeth, to which they give a yellowish color, and they are avoided in children up to 8 years of age. Common complications of tetracycline therapy are gastrointestinal disturbance due to alteration of the normal flora, predisposing to superinfection with tetracycline-resistant organisms and vaginal or oral candidiasis (thrush) due to the opportunistic yeast *Candida albicans*.

Dental staining limits use in children

## *Chloramphenicol*

Chloramphenicol has a simple nitrobenzene ring structure that can now be mass produced by chemical synthesis. It influences protein synthesis by binding to the 50S ribosomal subunit and blocking the action of peptidyl transferase, which prevents formation of the peptide bond essential for extension of the peptide chain. Its action is reversible in most susceptible species; thus, it is bacteriostatic. It has little effect on eukaryotic ribosomes, which explains its selective toxicity.

Chloramphenicol blocks peptidyl transferase

A broad-spectrum antibiotic, chloramphenicol, like tetracycline, has a wide range of activity against both aerobic and anaerobic species (see Table 13–1). Chloramphenicol is readily adsorbed from the upper gastrointestinal tract and diffuses readily into most body compartments, including the cerebrospinal fluid. It also permeates readily into mammalian cells and is active against obligate intracellular pathogens such as *Rickettsia* and *Chlamydia*.

Diffusion into body fluid compartments occurs readily

The major drawback to this inexpensive, broad-spectrum antimicrobial with almost ideal pharmacologic features is a rare but serious toxicity. Between 1 in 50,000 and 1 in 200,000 patients treated with even low doses of chloramphenicol have an idiosyncratic

Marrow suppression and aplastic anemia are serious toxicities reaction that results in aplastic anemia. The condition is irreversible and, before the advent of bone marrow transplantation, it was universally fatal. In high doses, chloramphenicol also causes a reversible depression of the bone marrow and, in neonates, abdominal, circulatory, and respiratory dysfunction. The inability of the immature infant liver to conjugate and excrete chloramphenicol aggravates this latter condition.

Use is sharply restricted

Chloramphenicol use is now restricted to treatment of rickettsial or ehrlichial infections in which tetracyclines cannot be used because of hypersensitivity or pregnancy. Its central nervous system (CNS) penetration and activity against anaerobes continue to lend support to its use in brain abscess. In some developing countries, chloramphenicol use is more extensive because of its low cost and proven efficacy in diseases such as typhoid fever and bacterial meningitis.

## *Macrolides*

Ribosomal binding blocks translocation

The macrolides, **erythromycin, azithromycin,** and **clarithromycin,** differ in the exact composition of a large 14- or 15-member ring structure. They affect protein synthesis at the ribosomal level by binding to the 50S subunit and blocking the translocation reaction. Their effect is primarily bacteriostatic. Macrolides, which are concentrated in phagocytes and other cells, are effective against some intracellular pathogens.

Erythromycin is active against Gram-positives and *Legionella*

Erythromycin, the first and still the most commonly used macrolide, has a spectrum of activity that includes most of the pathogenic Gram-positive bacteria and some Gram-negative organisms. The Gram-negative spectrum includes *Neisseria, Bordetella, Campylobacter,* and *Legionella,* but not the Enterobacteriaceae. Erythromycin is also effective against *Chlamydia* and *Mycoplasma.*

Azithromycin and clarithromycin have enhanced Gram-negative spectrum

Bacteria that have developed resistance to erythromycin are usually resistant to the newer macrolides azithromycin and clarithromycin as well. These newer agents have the same spectrum as erythromycin, with some significant additions. Azithromycin has quantitatively greater activity (lower MICs) against most of the same Gram-negative bacteria. Clarithromycin is the most active of the three against both Gram-positive and Gram-negative pathogens. Clarithromycin is also active against mycobacteria. In addition, both azithromycin and clarithromycin have demonstrated efficacy against *Borrelia burgdorferi,* the causal agent of Lyme disease and the protozoan parasite *Toxoplasma gondii*, which causes toxoplasmosis.

## *Clindamycin*

Spectrum is similar to macrolides with addition of anaerobes

Clindamycin is chemically unrelated to the macrolides but has a similar mode of action and spectrum. It has greater activity than the macrolides against Gram-negative anaerobes, including the important *Bacteroides fragilis* group. Although clindamycin is a perfectly adequate substitute for a macrolide in many situations, its primary use is in instances where anaerobes are or may be involved.

## *Oxazolidinones*

Activity against Gram-positive bacteria resistant to other agents

**Linezolid** is the most widely used of a new class of antibiotics that act by binding to the bacterial 50S ribosome. The exact mechanism is not known, but it does not involve peptide elongation or termination of translation. Oxazolidinones are clinically useful in pneumonia and other soft tissue infections, particularly those caused by resistant strains of staphylococci, pneumococci, and enterococci.

## *Streptogramins*

Useful against vancomycin-resistant enterococci

Quinupristin and dalfopristin are used in a fixed combination known as **quinupristin-dalfopristin** in a synergistic ratio. They inhibit protein synthesis by binding to different sites on the 50S bacterial ribosome; quinupristin inhibits peptide chain elongation, and dalfopristin interferes with peptidyl transferase. Their clinical use thus far has been limited to treatment of vancomycin-resistant enterococci.

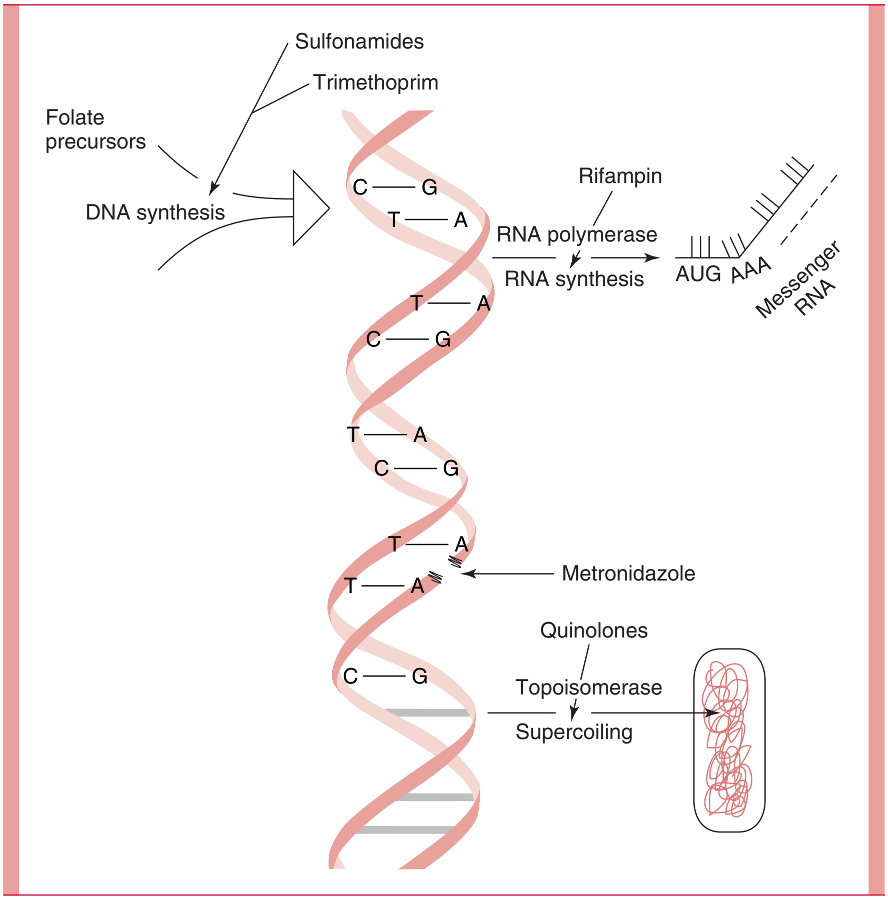


**FIGURE 13–4** Diagrammatic representation of antimicrobials acting on nucleic acids. Sulfonamides block the folate precursors of DNA synthesis, metronidazole inflicts breaks in the DNA itself, rifampin inhibits the synthesis of RNA from DNA by inhibiting RNA polymerase, and quinolones inhibit DNA topoisomerase and thus prevent the supercoiling required for the DNA to "fit" inside the bacterial cell.

## Inhibitors of Nucleic Acid Synthesis (Fig 13–4)

### *Quinolones*

The quinolones have a nucleus of two fused six-member rings that when substituted with fluorine become fluoroquinolones, which are now the dominant quinolones for treatment of bacterial infections. Among the fluoroquinolones, **ciprofloxacin, norfloxacin,** and **ofloxacin,** the addition of a piperazine ring and its methylation alter the activity and pharmacologic properties of the individual compound. The primary target of all quinolones is DNA topoisomerase (gyrase), the enzyme responsible for nicking, supercoiling, and sealing bacterial DNA during replication. Bacterial topoisomerases have four subunits, one or more of which are inhibited by the particular quinolone. The enhanced activity and lower frequency of resistance seen with the fluoroquinolones is attributed to binding at multiple sites on the enzyme. This greatly reduces the chance a single mutation can lead to resistance, which was a problem with the first quinolone, nalidixic acid, a single-binding site agent.

Fluorinated derivatives are now dominant

Inhibition of topoisomerase blocks supercoiling

The fluoroquinolones are highly active and bactericidal against a wide range of aerobes and facultative anaerobes. However, streptococci and *Mycoplasma* are only marginally susceptible, and anaerobes are generally resistant. Ofloxacin has significant activity against *Chlamydia,* whereas ciprofloxacin is particularly useful against *P. aeruginosa*. Fluoroquinolones has several favorable pharmacologic properties in addition to their broad spectrum. These include oral administration, low protein binding, good distribution to all body compartments, penetration of phagocytes, and a prolonged serum half-life that allows once- or twice-a-day dosing. Norfloxacin and ciprofloxacin are excreted by hepatic and renal routes, resulting in high drug concentrations in the bile and urine. Ofloxacin is excreted primarily by the kidney.

Fluoroquinolones have a broad spectrum, including *Pseudomonas*

Well distributed after oral administration

### *Folate Inhibitors*

Agents that interfere with synthesis of folic acid by bacteria have selective toxicity because mammalian cells are unable to accomplish this feat and use preformed folate from dietary sources. Folic acid is derived from *para*-aminobenzoic acid (PABA), glutamate, and a pteridine unit. In its reduced form, it is an essential coenzyme for the transport of one-carbon compounds in the synthesis of purines, thymidine, some amino acids, and, thus, indirectly of nucleic acids and proteins. The major inhibitors of the folate pathway are the sulfonamides, trimethoprim, *para*-aminosalicylic acid, and the sulfones.

Bacteria must synthesize folate that humans acquire in their diet

***Sulfonamides*** Sulfonamides are structural analogs of PABA and compete with it for the enzyme (dihydropteroate synthetase) that combines PABA and pteridine in the initial stage of folate synthesis. This blockage has multiple effects on the bacterial cells; the most important of these is disruption of nucleic acid synthesis. The effect is bacteriostatic, and the addition of PABA to a medium that contains sulfonamide neutralizes the inhibitory effect and allows growth to resume.

Competition with PABA disrupts nucleic acids

When introduced in the 1940s, sulfonamides had a very broad spectrum (staphylococci, streptococci, many Gram-negative bacteria) but resistance developed quickly, and this has restricted their use for systemic infections. Now their primary use is for uncomplicated urinary tract infections caused by members of the Enterobacteriaceae, particularly *Escherichia coli.* Sulfonamides are convenient for this purpose because they are inexpensive, well absorbed by the oral route, and excreted in high levels in the urine.

Major use is urinary tract infections

***Trimethoprim-Sulfamethoxazole*** Trimethoprim acts on the folate synthesis pathway but at a point after sulfonamides. It competitively inhibits the activity of bacterial dihydrofolate reductase, which catalyzes the conversion of folate to its reduced active coenzyme form. When combined with sulfamethoxazole, a sulfonamide, trimethoprim leads to a two-stage blockade of the folate pathway, which often results in synergistic bacteriostatic or bactericidal effects. This quality is exploited in therapeutic preparations that combine both agents in a fixed proportion designed to yield optimum synergy.

Dihydrofolate reductase inhibition is synergistic with sulfonamides

Trimethoprim-sulfamethoxazole (TMP-SMX) has a much broader and stable spectrum than either of its components alone; this includes most of the common pathogens, whether they are Gram-positive or Gram-negative, cocci or bacilli. Anaerobes and *P. aeruginosa* are exceptions. It is also active against some uncommon agents such as *Nocardia*. TMP-SMX is widely and effectively used in the treatment of urinary tract infections, otitis media, sinusitis, prostatitis, and infectious diarrhea, and it the agent of choice for pneumonia caused by *Pneumocystis carinii,* a fungus.

Activity against common bacteria and some fungi

## *Metronidazole*

Metronidazole is a nitroimidazole, a family of compounds with activity against bacteria, fungi, and parasites. The antibacterial action requires reduction of the nitro group under anaerobic conditions, which explains the limitation of its activity to bacteria that prefer anaerobic or at least microaerophilic growth conditions. The reduction products act on the cell at multiple points; the most lethal of these effects is induction of breaks in DNA strands.

Metronidazole is active against a wide range of anaerobes, including *Bacteroides fragilis*. Clinically, it is useful for any infection in which anaerobes may be involved. Because these infections are typically polymicrobial, a second antimicrobial (eg, $\beta$-lactam) is usually added to cover aerobic and facultative bacteria.

Action requires anaerobic conditions

## *Rifampin*

Rifampin binds to the $\beta$-subunit of DNA-dependent RNA polymerase, which prevents the initiation of RNA synthesis. This agent is active against most Gram-positive bacteria and selected Gram-negative organisms, including *Neisseria* and *Haemophilus* but not members of the Enterobacteriaceae. The most clinically useful property of rifampin is its antimycobacterial activity, which includes *Mycobacterium tuberculosis* and the other species that most commonly infect humans. Because resistance by mutation of the polymerase readily

Blocking of RNA synthesis occurs by binding to polymerase occurs, rifampin is combined with other agents in the treatment of active infections. It is used alone for chemoprophylaxis.

### Antimicrobics Acting on the Outer and Cytoplasmic Membranes

The polypeptide antimicrobics **polymyxin B** and **colistin** have a cationic detergent-like effect. They bind to the cell membranes of susceptible Gram-negative bacteria and alter their permeability, resulting in loss of essential cytoplasmic components and bacterial death. These agents react to a lesser extent with cell membranes of the host, resulting in nephrotoxicity and neurotoxicity. Their spectrum is essentially Gram-negative; they act against *P. aeruginosa* and other Gram-negative rods. Although these antimicrobics were used for systemic treatment in the past, their use is now limited to topical applications. They have an advantage; resistance to them rarely develops.

Binding to cytoplasmic membrane occurs

### Other Agents

Several other effective antimicrobics are in use almost exclusively for a single infectious agent or types of infections such as tuberculosis, urinary tract infections, and anaerobic infections. Where appropriate, these agents will be discussed in the relevant chapter. It is beyond the scope and intent of this book to provide comprehensive coverage of all available agents.

# ANTIVIRAL THERAPY

## GENERAL CONSIDERATIONS

Viruses are comprised of either DNA or RNA, a protein coat (capsid) and, in many, a lipid or lipoprotein envelope. The nucleic acid codes for enzymes involved in replication and for several structural proteins. Viruses use molecules (eg, amino acids, purines, pyrimidines) supplied by the cell and cellular structures (eg, ribosomes) for synthetic functions. Thus, one of the challenges in the development of antiviral agents is identification of the steps in viral replication that are unique to the virus and not used by the normal cell. Among the unique viral events are attachment, penetration, uncoating, RNA-directed DNA synthesis (reverse transcription), and assembly and release of the intact virion. Each of these steps may have complex elements with the potential for inhibition. For example, assembly of some virus particles requires a unique viral enzyme, protease, and this has led to the development of protease inhibitors.

Events in the cell unique to viral replication are the target

In some cases, antivirals do not selectively inhibit a unique replicative event but inhibit DNA polymerase. Inhibitors of this enzyme take advantage of the fact that the virus is synthesizing nucleic acids more rapidly than the cell, so there is relatively greater inhibition of viral than cellular DNA. In many acute viral infections, especially respiratory ones, the bulk of viral replication has already occurred when symptoms are beginning to appear. Initiating antiviral therapy at this stage is unlikely to make a major impact on the illness. For these viruses, immuno- or chemoprophylaxis, rather than therapy, is a more logical approach. However, many other viral infections are characterized by ongoing viral replication and do benefit from viral inhibition, such as human immunodeficiency virus (HIV) infection and chronic hepatitis B and C.

DNA polymerase is often inhibited

The principal antiviral agents in current use are discussed according to their modes of action. Their features are summarized in Table 13–2.

## SELECTED ANTIVIRAL AGENTS

### Inhibitors of Attachment

Attachment to a cell receptor is a virus-specific event. Antibody can bind to extracellular virus and prevent this attachment. However, although therapy with antibody is useful in prophylaxis, it has been minimally effective in treatment.

TABLE 13–2

**Summary of Antiviral Agents**

| Mechanism of Action | Antiviral Agent | Viral Spectrum[a] |
|---|---|---|
| Inhibition of viral uncoating, penetration | | |
| | Amantadine | Flu A |
| | Rimantadine | Flu A |
| Neuraminidase inhibition | | |
| | Oseltamivir | Flu A, Flu B |
| | Zanamivir | Flu A, Flu B |
| Inhibition of viral DNA polymerase | | |
| | Acyclovir | HSV, VZV |
| | Famciclovir | HSV, VZV |
| | Penciclovir | HSV |
| | Valacyclovir | HSV, VZV |
| | Ganciclovir | CMV, HSV, VZV |
| | Foscarnet | CMV, resistant HSV |
| | Cidofovir | CMV |
| | Trifluridine | HSV, VZV |
| Inhibition of viral reverse transcriptase | | |
| | Zidovudine | HIV |
| | Dideoxyinosine | HIV |
| | Dideoxycytidine | HIV |
| | Stavudine | HIV |
| | Lamivudine | HIV, HBV[b] |
| | Nevirapine | HIV |
| | Delavirdine | HIV |
| | Efavirenz | HIV |
| Inhibition of viral protease | | |
| | Saquinavir | HIV |
| | Indinavir | HIV |
| | Ritonavir | HIV |
| | Nelfinavir | HIV |
| | Lopinavir | HIV |
| Inhibition of viral protein synthesis | | |
| | Interferon $\alpha$ | HBV, HCV, HPV |
| Inhibition of viral RNA polymerase | | |
| | Ribavirin | RSV, HCV,[b] Lassa fever |
| Antisense inhibition of viral mRNA synthesis | | |
| | Fomivirsen | CMV |

[a] Flu A, influenza A; Flu B, influenza B; HSV, herpes simplex viruses; VZV, varicella-zoster virus; CMV, cytomegalovirus; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; RSV, respiratory syncytial virus; HPV, human papillomavirus.
[b] Used in combination with interferon.

## Inhibitors of Cell Penetration and Uncoating

**Rimantadine** differs from **amantadine** by the substitution of a methyl group for a hydrogen ion. These two amines inhibit several early steps in viral replication, including viral uncoating. They are extremely selective, with activity against only influenza A. In addition, they are effective in preventing influenza but are less useful in treatment of this viral infection due in part to the brief period of viral replication.

Amantadine and rimantadine are symmetrical amines, or acyclics, that inhibit early steps in replication

Effective only against influenza A viruses

### *Pharmacology and Toxicity*

Both amantadine and rimantadine are available only as oral preparations. The pharmacokinetics of the two agents is quite different. Amantadine is excreted by the kidney without being metabolized and its dose must be decreased in patients with impaired renal function. In contrast, rimantadine is metabolized by the liver and then excreted in the kidney and dosage adjustment for renal failure is not necessary.

Amantadine is excreted by the kidney

Rimantadine is metabolized by the liver

### *Treatment*

In healthy adults or children, amantadine and rimantadine show a slight but statistically significant improvement in symptoms compared to placebos or antipyretics. It has been assumed but not proved that these drugs are efficacious for treatment of influenza in elderly or other high-risk patients who may have more severe influenza. Influenza A strains resistant to these agents may appear rapidly in patients treated for clinical illness. Such strains can spread to patients receiving the drug prophylactically and can impair its efficacy as a preventive.

Viral resistance can appear rapidly

### *Prophylaxis*

The acyclics amantadine and rimantadine are approximately 70% effective in preventing influenza A illness when given daily during influenza outbreaks. Although illness is prevented or diminished, patients may still develop evidence of infection (ie, antibody), which is desirable because this antibody may provide some protection against future influenza A infection. These agents may be given alone or with vaccine. In the latter case, they may be given only until vaccine-induced antibody develops (eg, approximately 2 weeks) or they may be continued if a vaccine response is expected to be poor or marginal.

Useful in prophylaxis of influenza A

## Neuraminidase Inhibitors

**Oseltamivir** and **zanamivir** are new antivirals that selectively inhibit the neuraminidase of influenza A and B viruses. The neuraminidase cleaves terminal sialic acid from glycoconjugates and plays a role in the release of virus from infected cells. Zanamivir was the first approved neuraminidase inhibitor. It is given by oral inhalation using a specially designed device. Oseltamivir phosphate is the oral prodrug of oseltamivir, a drug comparable to zanamivir in antineuraminidase activity.

Treatment with either oseltamivir and zanamivir reduces influenza symptoms and shortens the course of illness by 1 to 1.5 days. The activity of these compounds against both influenza A and B offers an advantage over amantadine and rimantadine, which are active only against influenza A.

Neuraminidase inhibitors are effective in treatment and prophylaxis of influenza A and B viruses

## Inhibitors of Nucleic Acid Synthesis

At present, most antiviral agents are nucleoside analogs that are active against virus-specific nucleic acid polymerases or transcriptases and have much less activity against analogous host enzymes. Some of these agents serve as nucleic acid chain terminators after incorporation into nucleic acids.

### *Idoxuridine and Trifluorothymidine*

**Idoxuridine** (5-iodo-2′-deoxyuridine, IUdR) is a halogenated pyrimidine that blocks nucleic acid synthesis by being incorporated into DNA in place of thymidine and producing a nonfunctional molecule (ie, by terminating synthesis of the nucleic acid chain). It is phosphorylated by thymidine kinase to the active compound. Unfortunately, it inhibits both viral and cellular DNA synthesis, and the resulting host toxicity precludes systemic administration in humans. Idoxuridine can be used topically as effective treatment of herpetic infection of the cornea (keratitis). **Trifluorothymidine,** a related pyrimidine analog, is effective in treating herpetic corneal infections, including those that fail to respond to IUdR. It has largely replaced idoxuridine.

Idoxuridine and trifluorothymidine block DNA synthesis

## *Acyclovir*

This antiviral agent differs from the nucleoside guanosine by having an acyclic (hydroxyethoxymethyl) side chain. Acyclovir is unique in that it must be phosphorylated by thymidine kinase to be active, and this phosphorylation occurs only in cells infected by certain herpesviruses. Therefore, the compound is essentially nontoxic, because it is not phosphorylated or activated in uninfected host cells. Viral thymidine kinase catalyzes the phosphorylation of acyclovir to a monophosphate. From that point, host cell enzymes complete the progression to the diphosphate and finally the triphosphate.

Acyclovir is effective against herpesviruses that induce thymidine kinase

Activity of acyclovir against herpesviruses directly correlates with the capacity of the virus to induce a thymidine kinase. Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are the most active thymidine kinase inducers and are the most readily inhibited by acyclovir. Cytomegalovirus (CMV) induces little or no thymidine kinase and is not inhibited. Varicella-zoster and Epstein-Barr viruses are between these two extremes in terms of both thymidine kinase induction and acyclovir susceptibility.

Inhibits viral DNA polymerase and terminates viral DNA chain growth

Acyclovir triphosphate inhibits viral replication by competing with guanosine triphosphate and inhibiting the function of the virally encoded DNA polymerase. The selectivity and minimal toxicity of acyclovir is aided by its 100-fold or greater affinity for viral DNA polymerase than for cellular DNA polymerase. A second mechanism of viral inhibition results from incorporation of acyclovir triphosphate into the growing viral DNA chain. This causes termination of chain growth, because there is no 3′-hydroxy group on the acyclovir molecule to provide attachment sites for additional nucleotides. Resistant strains of HSV have been recovered from immunocompromised patients, including patients with acquired immunodeficiency syndrome (AIDS), and in most instances, resistance results from mutations in the viral thymidine kinase gene, rendering it inactive in phosphorylation. Resistance may also result from mutations in the viral DNA polymerase. Resistant virus has rarely, if ever, been recovered from immunocompetent patients, even after years of drug exposure.

Inhibits herpes viruses in blood

***Pharmacology and Toxicity*** Acyclovir is available in three forms: topical, oral, and parenteral. Topical acyclovir is rarely used. The oral form has low bioavailability (approximately 10%) but achieves concentrations in blood that inhibit HSV and to a lesser extent varicella-zoster virus (VZV). Intravenous acyclovir is used for serious HSV infection (eg, congenital), encephalitis, and VZV infection in immunocompromised patients. Because acyclovir is excreted by the kidney, the dosage must be reduced in patients with renal failure. CNS and renal toxicity have been reported in patients treated with prolonged high intravenous doses. Acyclovir is remarkably free of bone marrow toxicity, even in patients with hematopoietic disorders.

Effective against herpes and zoster

***Treatment and Prophylaxis*** Acyclovir is effective in the treatment of primary HSV mucocutaneous infections or for severe recurrences in immunocompromised patients. The agent is also useful in neonatal infectious herpes encephalitis, and it is also recommended for VZV infection in immunocompromised patients and varicella in older children or adults. Acyclovir is beneficial against herpes zoster in elderly patients or any patient with eye involvement. In patients with frequent severe genital herpes, the oral form is effective in preventing recurrences. Because it does not eliminate the virus from the host, it must be taken daily to be effective. Acyclovir is minimally effective in the treatment of recurrent genital or labial herpes in otherwise healthy individuals.

### *Valacyclovir, Famciclovir, and Penciclovir*

**Valacyclovir** is a prodrug of acyclovir that is better absorbed and therefore can be used in lower and less frequent dosage. Once absorbed, it becomes acyclovir. It is currently approved for use in HSV and VZV infections in immunocompetent adult patients. Dosage adjustment is necessary in patients with impaired renal function.

**Famciclovir** is similar to acyclovir in its structure and requirement for phosphorylation but differs slightly in its mode of action. After absorption, the agent is converted to penciclovir, the active moiety, which is also a competitive inhibitor of a guanosine triphosphate. However, it does not irreversibly terminate DNA replication. Famciclovir is currently approved for treatment of HSV and VZV infections. **Penciclovir,** itself, is approved for topical treatment of recurrent herpes labialis.

Agents that are similar to or become acyclovir after absorption are available

### *Ganciclovir*

Ganciclovir (DHPG), a nucleoside analog of guanosine, differs from acyclovir by a single carboxyl side chain. This structural change confers approximately 50 times more activity against CMV. Acyclovir has low activity against CMV, because it is not well phosphorylated in CMV-infected cells due to the absence of the gene for thymidine kinase in CMV. However, ganciclovir is active against CMV and does not require thymidine kinase for phosphorylation. Instead, another viral-encoded phosphorylating enzyme (UL97) is present in CMV-infected cells that is capable of phosphorylating ganciclovir and converting it to the monophosphate. Then cellular enzymes convert it to the active compound, ganciclovir triphosphate, which inhibits the viral DNA polymerase.

Ganciclovir does not require viral thymidine kinase for phosphorylation

Oral ganciclovir is available but is inferior to the intravenous form. Oral valganciclovir, a prodrug of ganciclovir, has improved bioavailability and is equivalent to the intravenous form. Toxicity frequently limits therapy. Neutropenia, which is usually reversible, may occur early but often develops during later therapy. Discontinuation of therapy is necessary in patients whose neutrophils do not increase during dosage reduction or in response to cytokines. Thrombocytopenia (platelet count $<$20,000/mm$^3$) occurs in approximately 15% of patients.

Neutropenia and thrombocytopenia limit use

***Clinical Use*** Administration of ganciclovir is indicated for the treatment of active CMV infection in immunocompromised patients, but other herpesviruses (particularly HSV-1, HSV-2, and VZV) are also susceptible. Because AIDS patients with severe CMV infection frequently have concurrent illnesses caused by other herpesviruses, treatment with ganciclovir may benefit associated HSV and VZV infections.

***Resistance*** After several months of continuous ganciclovir therapy for treatment of CMV, between 5 and 10% of AIDS patients excrete resistant strains of CMV. In virtually all isolates, there is a mutation in the phosphorylating gene, and in a lesser number there may also be a mutation in the viral DNA polymerase. The great majority of these strains remains sensitive to foscarnet, which may be used as alternate therapy. If only a UL97 mutation is present, the strains remain susceptible to cidofovir; however, most of the strains with a ganciclovir-induced mutation in DNA polymerase are cross-resistant to cidofovir. Many clinicians tend to assume that when a patient with CMV retinitis has progression of the disease during treatment, viral resistance has developed. Progression of CMV disease during treatment is probably the result of many factors, only one of which is the susceptibility of the CMV strain to the drug. Blood and tissue concentrations of ganciclovir, penetration of ganciclovir into the retinal tissue, and the host immune response probably play important roles in determining when clinical progression of CMV disease occurs. Ganciclovir resistance is beginning to be noted in transplant recipients, especially those requiring prolonged treatment.

CMV mutant resistance increases with continuous therapy

## Inhibitor of Viral RNA Synthesis: Ribavirin

**Ribavirin** is another analog of the nucleoside guanosine. Unlike acyclovir, which replaces the ribose moiety with an hydroxymethyl acyclic side chain, ribavirin differs from guanosine in that the base ring is incomplete and open. Like other purine nucleoside analogs, ribavirin must be phosphorylated to mono-, di-, and triphosphate forms. It is active against a broad range of viruses in vitro, but its in vivo activity is limited. The mechanism of the antiviral effect of ribavirin is not as clear as that of acyclovir. The triphosphate is an inhibitor of RNA polymerase and it also depletes cellular stores of guanine by inhibiting inosine monophosphate dehydrogenase, an enzyme important in the synthetic pathway of guanosine. Still another mode of action is by decreasing synthesis of the mRNA 5′ cap because of interference with both guanylation and methylation of the nucleic acid base.

Ribavirin has several modes of action

Aerosol administration enables ribavirin to reach concentrations in respiratory secretions up to ten times greater than necessary to inhibit viral replication and substantially higher than those achieved with oral administration. Problems encountered with aerosolized ribavirin include precipitation of the agent in tubing used for administration and exposure of health care personnel.

Ribavirin is somewhat beneficial if given early by aerosol to infants who are infected with respiratory syncytial virus. Oral and intravenous forms have been used for patients with Lassa fever, although studies have been limited. In a recent trial of hantavirus treatment, ribavirin was ineffective. The oral form has limited activity against hepatitis C as monotherapy but provides additional benefit when combined with interferon alpha. A reversible anemia has been associated with oral administration of ribavirin.

Ribavirin is active against respiratory syncytial virus, Lassa fever virus, and hepatitis C

## Inhibitors of HIV

### *Nucleoside Reverse Transcriptase Inhibitors*

***Azidothymidine*** Azidothymidine (AZT), a nucleoside analog of thymidine, inhibits the reverse transcriptase of HIV. As with other nucleosides, AZT must be phosphorylated; host cell enzymes carry out the process. The basis for the relatively selective therapeutic effect of AZT is that HIV reverse transcriptase is more than 100 times more sensitive to AZT than is host cell DNA polymerase. Nonetheless, toxicity frequently occurs.

AZT was the first useful treatment for HIV infection but now is recommended for use only in combination with other inhibitors of HIV replication (eg, lamivudine and protease inhibitors). Toxicity includes malaise, nausea, and bone marrow toxicity. All hematopoietic components may be depressed but usually reverse with discontinuation of the drug or dose reduction. Resistance is associated with one or more mutations in the HIV reverse transcriptase gene.

AZT is now used only in combination therapy

***Didanosine and Zalcitabine*** Didanosine (ddI, dideoxyinosine) and zalcitabine (ddC, dideoxycytidine) are nucleoside analogs that inhibit HIV replication. Following intracellular phosphorylation by host enzymes to their active triphosphate form, they block viral replication by inhibiting viral reverse transcriptase, like zidovudine. Serious adverse effects of treatment include peripheral neuropathy with either ddI or ddC, and pancreatitis with ddI; both conditions are dose related. Dose reduction is required for impaired renal function. As with other anti-HIV drugs, these agents should be used only in combination with one or two other anti-HIV drugs to limit the development of resistance and to enhance antiviral effect.

ddI and ddC are always used in combination with other anti-HIV drugs

***Stavudine*** Stavudine (D4T) is another nucleoside analog that inhibits HIV replication. D4T is phosphorylated by cellular enzymes to an active triphosphate form that interferes with viral reverse transcriptase, and it also terminates the growth of the chain of viral nucleic acid. D4T is well absorbed and has a high bioavailability. Adverse effects include headache, nausea and vomiting, asthenia, confusion, and elevated serum transaminase and creatinine kinase. A painful sensory peripheral neuropathy that appears to be dose related has also been noted. Dose reduction is required for impaired renal function. D4T should be used only in combination with other anti-HIV agents.

D4T is a reverse transcriptase inhibitor that also terminates chain growth

***Lamivudine*** Lamivudine (3TC), another reverse transcriptase inhibitor, is a comparatively safe and usually well-tolerated agent and is used in combination with AZT or other

3TC suppresses development of AZT resistance nucleoside analogs. AZT and 3TC have a unique interaction; 3TC suppresses the development and persistence of AZT resistance mutations. When combined with interferon alpha, 3TC is also useful for treating hepatitis B.

### *Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)*

Compounds that are not nucleoside analogs also inhibit HIV reverse transcriptase. Several compounds, e.g., nevirapine, delavirdine, and efavirenz, have been evaluated alone or in combination with other nucleosides. These compounds are very active against HIV-1, do not require cellular enzymes to be phosphorylated, and bind to essentially the same site on reverse transcriptase. They are active against both AZT-resistant and AZT-sensitive isolates. In addition, most of these compounds do not inhibit human DNA polymerase and are not cytotoxic at concentrations required for effective antiviral activity; therefore, they are relatively nontoxic. Unfortunately, drug resistance readily emerges with even single passage of virus in the presence of drug in vitro and in vivo. Thus, NNRTIs should only be used in combination regimens with other drugs active against HIV.

NNRTIs are often active against AZT-resistant strains

Rapid development of drug resistance occur when NNRTIs are used alone

### *Protease Inhibitors*

The newest agents that inhibit HIV are the protease inhibitors. These agents block the action of the viral-encoded enzyme protease, which cleaves polyproteins to produce structural proteins. Inhibition of this enzyme leads to blockage of viral assembly and release. The protease inhibitors are potent suppressors of HIV replication in vitro and in vivo, particularly when combined with other antiretroviral agents.

In late 1995, **saquinavir** was the first protease inhibitor to receive approval. **Ritonavir, indinavir,** and **nelfinavir** are other potent protease inhibitors that have since been released. These drugs may cause hepatotoxicity and all agents inhibit P450, resulting in important drug interactions. Because drug resistance develops to all protease inhibitors, they should not be used alone without other anti-HIV drugs.

Protease inhibitors block viral-encoded proteases

Used in combination with other anti-HIV drugs

## Nucleotide Analogs: Cidofovir

In recent years a new series of antiviral compounds, the nucleotide analogs, have been developed. The best known example of this class of compounds is **cidofovir.** This compound mimics a monophosphorylated nucleotide by having a phosphonate group attached to the molecule. This appears to the cell as a nucleoside monophosphate, or nucleotide, and cellular enzymes then add two phosphate groups to generate the active compound. In this form, the drug inhibits both viral and cellular nucleic acid polymerases but selectivity is provided by its higher affinity for the viral enzyme.

Nucleotide analogs do not require phosphorylation, or activation, by a viral-encoded enzyme and remain active against viruses that are resistant due to mutations in codons for these enzymes. Resistance can develop with mutations in the viral DNA polymerase, UL54. An additional feature of cidofovir is a very prolonged half-life, due to slow clearance by the kidneys.

Cidofovir inhibits viral DNA polymerase

Cidofovir is approved for intravenous therapy of CMV retinitis, and maintenance treatment may be given as infrequently as every 2 weeks. Nephrotoxicity is a serious complication of cidofovir treatment, and patients must be monitored carefully for evidence of renal impairment.

## Other Antiviral Agents

### *Foscarnet*

Foscarnet, also known as phosphonoformate, is a pyrophosphate analog that inhibits viral DNA polymerase by blocking the pyrophosphate-binding site of the viral DNA polymerase and preventing cleavage of pyrophosphate from deoxyadenosine triphosphate. This action is relatively selective; CMV DNA polymerase is inhibited at concentrations less than 1% of that required to inhibit cellular DNA polymerase. Unlike such nucleosides as acyclovir and ganciclovir, foscarnet does not require phosphorylation to be an active inhibitor of viral DNA polymerases. This biochemical fact becomes especially important with regard to viral resistance, because the principal mode of viral resistance to nucleoside analogs is a mutation that eliminates phosphorylation of the drug in virus-infected cells. Thus, foscarnet can usually be used to treat patients with ganciclovir-resistant CMV and acyclovir-resistant HSV. Excretion is entirely renal without a hepatic component, and dosage must be decreased in patients with impaired renal function.

Foscarnet inhibits viral DNA polymerases

Effective against resistant CMV and HSV

### *Interferons*

Interferons are host cell–encoded proteins synthesized in response to double-stranded RNA that circulate to protect uninfected cells by inhibiting viral protein synthesis. Ironically, interferons harvested in tissue culture were the first antiviral agents, but their clinical activity was disappointing. Recombinant DNA techniques now allow relatively inexpensive large-scale production of interferons by bacteria and yeasts.

Recombinant DNA techniques allow large-scale production

Interferons inhibit viral protein synthesis

**Interferon alpha** is beneficial in the treatment of chronic active hepatitis B and C infection, although its efficacy is often transient. Combinations of interferon alpha with 3TC, famciclovir, and other nucleosides are being evaluated for treatment of hepatitis B. Interferon alpha is given for 6 to 12 months to treat chronic hepatitis C disease, and combination with ribavirin usually produces improved results. Topical interferon application is beneficial in the treatment of human papilloma virus infections. Interferons cause symptomatic systemic toxicity, (eg, fever, malaise), partly because of their effect on host cell protein synthesis.

Interferon alpha is combined with ribavirin to treat chronic hepatitis C

### *Fomivirsen*

Fomivirsen, the first antisense compound to be approved for use in human infection, is a synthetic oligonucleotide, complementary to and presumably inhibiting a coding sequence in CMV messenger RNA (mRNA). The major immediate early transcriptional unit of CMV encodes several proteins responsible for regulation of viral gene expression. Presumably, fomivirsen inhibits production of these proteins. In this agent, oligonucleotide phosphorothioate linkages replace the usual nucleases. Fomivirsen, which exhibits greater antiviral activity than ganciclovir on a molar basis, is approved for the local (intravitreal) therapy of CMV retinitis in patients who have failed other therapies.

Fomivirsen inhibits CMV mRNA

## ANTIVIRAL RESISTANCE

Viral genomes and their replication, as well as the mechanisms of action of the available antiviral agents, have been intensively studied. Accordingly, an understanding of resistance to antiviral drugs has evolved; investigation of resistance mechanisms has shed light on the function of specific viral genes. For example, it has become clear that a common mechanism of resistance to nucleosides (eg, acyclovir, ganciclovir) by herpesviruses are mutations in the viral-induced enzyme responsible for phosphorylating the nucleoside. For herpes simplex virus, this is thymidine kinase, and for CMV, this gene is designated UL97.

Herpesviruses often develop resistance by mutations in phosphorylation

Genetic alterations (ie, mutations or deletions) are the basis for antiviral resistance. The likelihood of resistant mutants results from at least four functions:

1. **Rate of viral replication.** Herpesviruses, especially CMV and VZV do not replicate as rapidly as HIV and hepatitis B and C. Higher rates of replication are associated with higher rates of spontaneous mutations.
2. **Selective pressure of the drug.** The selective pressure increases the probability of mutations to the point that virus replication is substantially reduced.
3. **Rate of viral mutations.** In addition to viral replication, the rate of mutations differs among different viruses. In general, single-stranded RNA viruses (eg, HIV, influenza) have more rapid rates of mutation than double-stranded DNA viruses (eg, herpesviruses).

4. **Rates of mutation in differing viral genes.** For example, within the herpesviruses, the genes for phosphorylating nucleosides (eg, UL97) are more susceptible to mutation than the viral DNA polymerase.
   Resistance to antivirals may be detected in several ways:

   - **Phenotypic.** This is the traditional method of growing virus in tissue culture in medium containing increasing concentrations of an antiviral agent. The concentration of the agent that reduces viral replication by 50% is the end point; it is referred to as the inhibitory concentration ($IC_{50}$). The $IC_{50}$ of resistant virus is higher than that of susceptible virus. The degree of viral replication is obtained by counting viral plaques (ie, equivalent to viral "colonies") or by measuring viral antigen or nucleic acid concentration. Unfortunately, phenotypic assays are very time-consuming, requiring days to weeks for completion. $IC_{50}$ values increase as the percent of the viral population with the mutation increases.

Phenotypic resistance is detected by in vitro methods

   - **Genotypic.** When the exact mutation or deletion responsible for antiviral resistance is known, it is possible to sequence the viral gene or detect it with restriction enzyme patterns. These tests are rapid but require knowledge of the expected mutation, and they do not provide quantitation of the percent of the viral population harboring the mutation. If only 1 or 5% of the population has the mutation, this result may not be clinically significant when compared to a virus population that is 90% mutated.

Genotypic = molecular detection of expected mutation

   - **Viral quantitation in response to treatment.** Various methods of quantitating virus (eg, culture, polymerase chain reaction, antigen assay) provide a means of assessing the decline of viral titer in response to treatment with an antiviral agent. These assays are rapid and do not require knowledge of the expected mutation. If no decline occurs despite adequate dosage and compliance, viral resistance may be responsible. Likewise, if viral titer initially decreases but subsequently recurs and/or increases, then resistance may have developed.

No reduction or increase in patient's viral burden suggests development of resistant mutants

## ADDITIONAL READING

Balfour HH Jr. Antiviral drugs. *N Engl J Med* 1999;340:1255–1268. An excellent review of antivirals other than those used to treat HIV infections.

Hardman JG, Limbird LE, eds. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 10th ed. New York: McGraw-Hill; 2001. A standard reference text with excellent sections on antibiotics and chemotherapy.

Mandell GL, Bennett JE, Dolin R, eds. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases,* 5th ed. Philadelphia: Churchill Livingstone; 2002. This reference work discusses the mechanisms and clinical use of each antimicrobic in an individual chapter.

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CHAPTER 14

# Antimicrobial Resistance

KENNETH J. RYAN

The continuing success of antimicrobial therapy depends on keeping ahead of the ability of the microorganisms to develop resistance to antimicrobics. At times, resistance seems to occur at a rate equal to that of the development of new antimicrobics. The nature of resistance and the mechanisms bacteria use to achieve it are the subject of this chapter. The ways in which resistance affect medical practice and the way in which laboratory tests are used to guide clinicians through the uncertainties of modern treatment are also considered.

## SUSCEPTIBILITY AND RESISTANCE

Deciding whether any bacterium should be considered susceptible or resistant to any antimicrobic involves an integrated assessment of in vitro activity, pharmacologic characteristics, and clinical evaluation. Any agent approved for clinical use has demonstrated in vitro its potential to inhibit the growth of some target group of bacteria at concentrations that can be achieved with acceptable risks of toxicity. That is, the minimal inhibitory concentration (MIC) can be comfortably exceeded by doses tolerated by the patient. Use of the antimicrobic in animal models and then human infections must have also demonstrated a therapeutic response. Because the influence of antimicrobics on the natural history of different categories of infection (eg, pneumonia, meningitis, diarrhea) varies, extensive clinical trials must include both a range of bacterial species and infected sites. These studies are important to determine whether what should work actually does work and, if so, to define the parameters of success and failure.

MICs must be below achievable blood levels

Clinical experience must validate in vitro data

Once these factors are established, the routine selection of therapy can be based on known or expected characteristics of organisms and pharmacologic features of antimicrobics. With regard to organisms, use of the term **susceptible** (sensitive) implies that their MIC is at a concentration attainable in the blood or other appropriate body fluid (eg, urine) using the usually recommended doses. **Resistant,** the converse of susceptible, implies that the MIC is not exceeded by normally attainable levels. As in all biological systems, the MIC of some organisms lies in between the susceptible and resistant levels. Borderline strains are called **intermediate, moderately sensitive,** or **moderately resistant,** depending on the exact values and conventions of the reporting system. Antimicrobics may be used to treat these organisms but at increased doses, perhaps to reach body compartments where pathogens are concentrated. For example, nontoxic antimicrobics such as the penicillins and cephalosporins can be administered in massive doses and may thereby inhibit some pathogens that would normally be considered resistant in vitro.

Susceptible bacteria are inhibited at achievable nontoxic levels, resistant strains are not

Borderline isolates are called intermediate

Furthermore, in urinary infections, urine levels of some antimicrobics may be very high, and organisms that are seemingly resistant in vitro may be eliminated.

Pharmacologic properties such as absorption, distribution, and metabolism affect the usefulness of antimicrobics

Important pharmacologic characteristics of antimicrobics include dosage as well as the routes and frequency of administration. Other characteristics include whether the agents are absorbed from the upper gastrointestinal tract, whether they are excreted and concentrated in active form in the urine, whether they can pass into cells, whether and how rapidly they are metabolized, and the duration of effective antimicrobial levels in blood and tissues. Most agents are bound to some extent to serum albumin, and the protein-bound form is usually unavailable for antimicrobial action. The amount of free to bound antibiotic can be expressed as an equilibrium constant, which varies for different antibiotics. In general, high degrees of binding lead to more prolonged but lower serum levels of an active antimicrobic after a single dose.

## LABORATORY CONTROL OF ANTIMICROBIAL THERAPY

Bacteria are tested against antimicrobics over a range of concentrations

A unique feature of laboratory testing in microbiology is that the susceptibility of the isolate of an individual patient can be tested against a battery of potential antimicrobics. These tests are built around the common theme of placing the organism in the presence of varying concentrations of the antimicrobic in order to determine the MIC. The methods used are standardized, including a measured inoculum of the bacteria and the growth conditions (eg, medium, incubation, time).

Final selection of therapy considers susceptibility, pharmacology, and clinical experience

Bactericidal action is required for infections such as endocarditis

In selecting therapy, the results of laboratory tests cannot be considered by themselves, but must be examined with information about the clinical pharmacology of the agent, the cause of the disease, the site of infection, and the pathology of the lesion. These factors must all be taken into account when selecting the appropriate antimicrobic from those to which the organism has been reported as susceptible. If the agent cannot reach the site of infection, it will be ineffective. For example, the agent must reach the subarachnoid space and cerebrospinal fluid in the case of meningitis. Similarly, therapy may be ineffective for an infection that has resulted in abscess formation unless the abscess is surgically drained. In some instances (eg, bacterial endocarditis, agranulocytosis), it is necessary to use a bactericidal agent. Previous clinical experience is also critical. In typhoid fever, for instance, chloramphenicol is effective and aminoglycosides are not, even though the typhoid bacillus may be susceptible to both in vitro. This finding appears to result from the failure of aminoglycosides to achieve adequate concentrations inside infected cells.

### Dilution Tests

MIC is the lowest concentration that inhibits growth

Dilution tests determine the MIC directly by using serial dilutions of the antimicrobic in broth that span a clinically significant range of concentrations. The dilutions are prepared in tubes or microdilution wells, and by convention, they are doubled using a base of 1 μg/mL (0.25, 0.5, 1, 2, 4, 8, and so on). The bacterial inoculum is adjusted to a concentration of $10^5$ to $10^6$ bacteria/mL and added to the broth. After incubation overnight (or other defined time), the tubes are examined for turbidity produced by bacterial growth. The first tube in which visible growth is absent (clear) is the MIC for that organism (Fig 14–1).

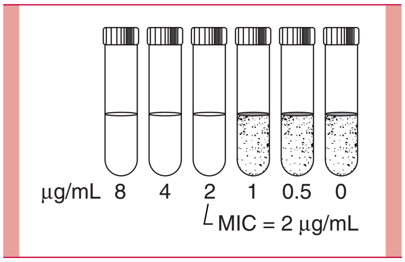



FIGURE 14–1
Broth dilution susceptibility test. The stippled tubes represent turbidity produced by bacterial growth. The MIC is 1.0 μg/mL.

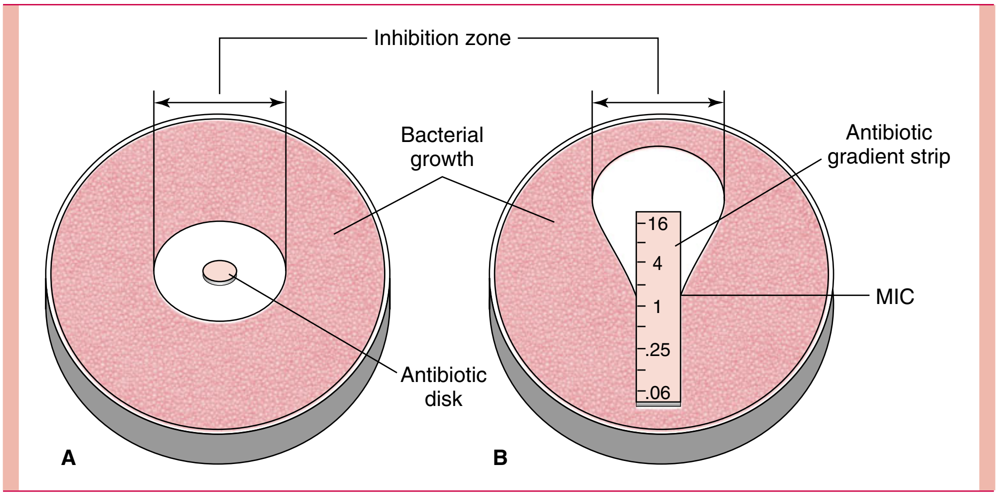



FIGURE 14–2
Diffusion tests. **A.** Disk diffusion. The diameter of the zone of growth inhibition around a disk of fixed antimicrobial content is inversely proportional to the minimum inhibitory concentration (MIC) for that antimicrobic. The larger the zone, the lower the MIC. **B.** The E test. A strip containing a gradient of antimicrobial content creates an elliptical zone of inhibition. The conditions are empirically adjusted so that the MIC is marked where the growth intersects the strip.

## Diffusion Tests

Antimicrobic diffuses into agar from disks to produce a circular concentration gradient/24

The diameter of the inhibition zone around the disk is a measure of the MIC

In diffusion testing, the inoculum is seeded onto the surface of an agar plate, and filter paper disks containing defined amounts of antimicrobics are applied. While the plates are incubating, the antimicrobic diffuses into the medium to produce a circular gradient around the disk. After incubation overnight, the size of the zone of growth inhibition around the disk (Fig 14–2A) can be used as an indirect measure of the MIC of the organism. It is also influenced by the growth rate of the organism, the diffusibility of the antimicrobic, and some technical factors. In the United States, a standardized diffusion procedure accounts for these factors and includes recommendations for interpretation. The diameters of the zones of inhibition obtained with the various antibiotics are converted to "susceptible," "moderately susceptible," and "resistant" categories by referring to a table. This method is convenient and flexible for rapidly growing aerobic and facultative bacteria such as the Enterobacteriaceae, *Pseudomonas,* and staphylococci. Another diffusion procedure uses gradient strips to produce elliptical zones that can be directly correlated with the MIC. This method, the E test (Fig 14–2B), can also be applied to slow-growing, fastidious, and anaerobic bacteria.

## Automated Tests

Automated tests read endpoints of broth dilution tests in a few hours

Instruments are now available that carry out rapid, automated variants of the broth dilution test. In these systems the bacteria are incubated with the antimicrobic in specialized modules that are read automatically every 15 to 30 minutes. The multiple readings and the increased sensitivity of determining endpoints by turbidimetric or fluorometric analysis makes it possible to generate MICs in as little as 4 hours. In laboratories with sufficient volume, these methods are no more expensive than manual methods, and the rapid results have enhanced potential to influence clinical outcome, particularly when interfaced with computerized hospital information systems.

## Molecular Testing

Molecular methods detect resistance genes

The molecular techniques of nucleic acid hybridization, sequencing, and amplification (see Chapter 15) have been applied to the detection and study of resistance. The basic strategy is to detect the resistance gene rather than measure the phenotypic expression of resistance. These methods offer the prospect of automation and rapid results, but as with most molecular methods, are not yet practical for routine use. Their application will also have to take consideration of the fact that they will be limited to known genes and that phenotypic expression is the "bottom line."

## Bactericidal Testing

Quantitation of the bactericidal effect determines the MBC

The above methods do not distinguish between inhibitory and bactericidal activity. To do so requires quantitative subculture of the clear tubes in the broth dilution test and comparison of the number of viable bacteria at the beginning and end of the test. The least amount required to kill a predetermined portion of the inoculum (usually 99.9%) is called the **minimal bactericidal concentration (MBC).** Direct bactericidal testing is important in the initial characterization and clinical evaluation of antimicrobics but is rarely needed in individual cases. Most of the antimicrobics used for acute and life-threatening infections (eg, $\beta$-lactams, aminoglycosides) act by bactericidal mechanisms.

## Antimicrobial Assays

Pharmacologic monitoring is necessary in some situations

For antimicrobics with toxicity near the therapeutic range, monitoring the concentration in the serum or other appropriate body fluid is sometimes necessary. Therapeutic monitoring may also be required when the patient's pharmacologic handling of the agent is unpredictable, as in renal failure. A variety of biologic, immunoassay, and chemical procedures have been developed for this purpose.

# BACTERIAL RESISTANCE TO ANTIMICROBICS

Resistance has eroded the effectiveness of many agents

The seemingly perfect nature of antimicrobics, originally hailed as "wonder drugs," has been steadily eroded by the appearance of strains resistant to their action. This resistance may be inherent to the organism or appear in a previously susceptible species by mutation or the acquisition of new genes. The mechanisms by which bacteria develop resistance and how this resistance is spread are of great interest for the continued use of current agents and to develop strategies for the development of new antimicrobics. The following sections discuss the biochemical mechanisms of resistance, how resistance is genetically controlled, and how resistant strains survive and spread in our society. How these features relate to the antimicrobic groups is summarized in Table 14–1 and further discussed in the chapters on specific bacteria (see Chapters 16 to 32).

Resistance and virulence are separate properties

Antimicrobial resistance has survival value for the organism, and its expression in the medical setting requires that virulence be retained despite the change that mediates resistance. There are no direct connections between resistance and virulence. Resistant bacteria may have increased opportunities to produce disease, but the disease is the same as that produced by the susceptible bacteria's counterpart.

## Mechanisms of Resistance

The major mechanisms of bacterial resistance are (1) accumulation barriers to an antimicrobic due to impermeability or active efflux; (2) alterations of an antimicrobic target, which render it insusceptible; and (3) inactivation of an antimicrobic by an enzyme produced by the microorganism. Changes in metabolic pathways can also translate into resistance in a few antimicrobic–organism combinations.

### *Accumulation Barriers (Fig 14–3)*

Cell wall and outer membrane are barriers to antimicrobics

Outer membrane protein porins restrict access to interior

An effective antimicrobic must enter the bacterial cell and achieve concentrations sufficient to act on its target. The cell wall, particularly the outer membrane, of Gram-negative bacteria presents a formidable barrier for access to the interior of the cell. Outer membrane protein porin channels may allow penetration depending on the size, charge, degree of hydrophobicity, or general molecular configuration of the molecule. This is a major reason for inherent resistance to antimicrobics, but these transport characteristics may change even in typically susceptible species due to mutations in the porin proteins.

TABLE 14–1

**Features of Bacterial Resistance to Antimicrobial Agents**

| Antimicrobic | Mechanism[a]: Altered Accumulation (AA) | Mechanism[a]: Altered Target (AT) | Mechanism[a]: Enzymatic Inactivation (EI) | Emerging Resistance[b] (Organism/Antimicrobic/Mechanism) |
|---|---|---|---|---|
| *β*-lactams | Variable outer membrane[c] penetration | Mutant and new PBPs | *β*-lactamases | *Staphylococcus aureus*/penicillin/EI<br>*S. aureus*/methicillin/AT<br>*Streptococcus pneumoniae*/penicillin/AT<br>*Haemophilus in uenzae* /ampicillin/AT, EI<br>*Neisseria gonorrhoeae*/penicillin/AT, EI<br>*Pseudomonas aeruginosa*/ceftazidime/AA<br>*Klebsiella, Enterobacter*/third-generation cephalosporins/EI |
| Glycopeptides | – | Amino acid substitution | – | *Enterococcus*/vancomycin/AT<br>*S. aureus*/vancomycin (rare) |
| Aminoglycosides | Oxidative transport required | Ribosomal binding site mutations | Adenylases, acetylases, phosphorylases | *Klebsiella, Enterobacter*/gentamicin/EI<br>*P. aeruginosa*/gentamicin/AA |
| Macrolides, clindamycin | Minimal outer membrane[c] penetration, efflux pump | Methylation of rRNA | Phosphotransferase, esterase | *Bacteroides fragilis*/clindamycin/AT<br>*S. aureus*/erythromycin/AT |
| Chloramphenicol | – | – | Acetyltransferase | *Salmonella*/chloramphenicol/EI |
| Tetracycline | Efflux pump | New protein protects ribosome site | – | |
| Fluoroquinolones | Efflux pump, permeability mutation | Mutant topoisomerase | – | *Escherichia coli*/ciprofloxacin/AT<br>*P. aeruginosa*/ciprofloxacin/AT |
| Rifampin | – | Mutant RNA polymerase | – | *Mycobacterium tuberculosis*[d]/rifampin/AT<br>*Neisseria meningitidis*/rifampin/AT |
| Folate inhibitors | – | New dihydropteroate synthetase, altered dihydrofolate reductase | – | Enterobacteriaceae/sulfonamides/AT |

[a] Only primary mechanisms of resistance are listed.
[b] A highly selective list of resistance emergence that has altered or threatens a major clinical use of the agent.
[c] Outer membrane of Gram-negative bacteria.
[d] See Chapter 28.
*Abbreviations:* PBP, penicillin-binding protein.

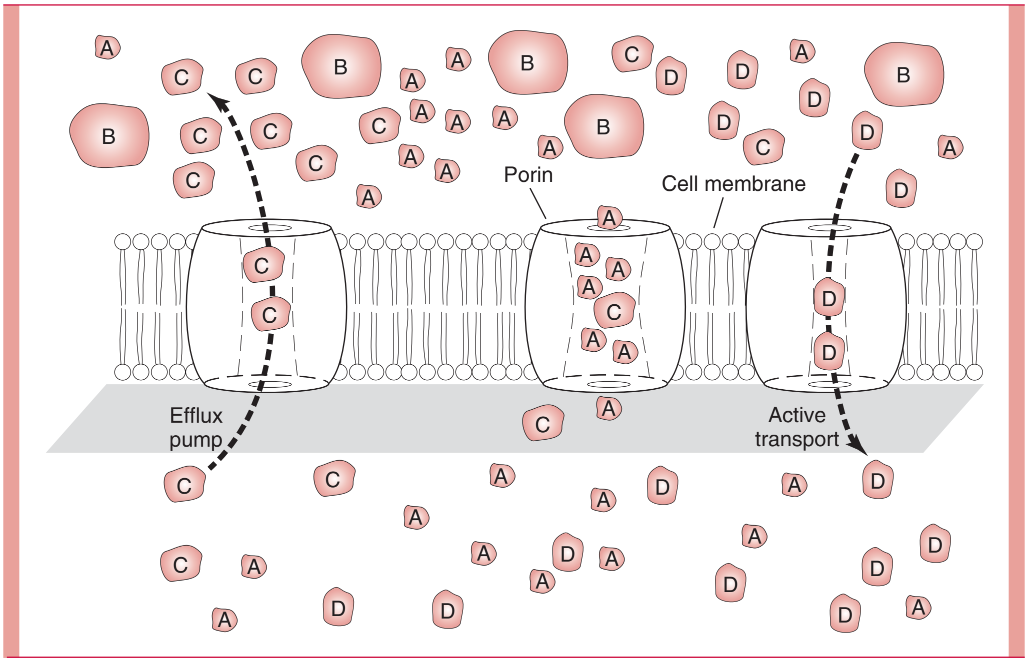



FIGURE 14–3
Diagrammatic representation of accumulation barrier resistance. A, B, C, and D molecules are external to the cell wall. A molecules pass through and remain inside the cell, B molecules are unable to pass because they cannot fit through any of the channels, C molecules pass through but are pushed back out by an efflux pump, and D molecules must be pulled through the wall by an active process.

For example, strains of *Pseudomonas aeruginosa* commonly develop resistance to imipenem due to loss of the outer membrane protein most important in its penetration.

Drugs are actively transported in and out of cells

As mentioned above, some antimicrobics must be actively transported into the cell. Bacteria such as streptococci, enterococci, and anaerobes, which lack the necessary oxidative pathways for transport of aminoglycosides, are resistant. Conversely, some antimicrobics are actively transported out of the cell. A number of bacterial species have energy-dependent efflux mechanisms that pump either tetracyclines or fluoroquinolones from the cell.

## *Altered Target (Fig 14–4)*

Binding affinity for enzymes and ribosomes can change

Once in the cell, antimicrobics act by binding and inactivating their target, which is typically a crucial enzyme or ribosomal site. If the target is altered in a way that decreases its affinity for the antimicrobic, the inhibitory effect will be proportionately decreased. Substitution of a single amino acid at a certain location in a protein can alter its binding to the antimicrobic without affecting its function in the bacterial cell.

Multiple binding sites reduces chances for resistance

If an alteration at a single site of the target does not render it susceptible to the antimicrobic, mutation to resistance can occur in a single step, even during therapy. This occurred with the early aminoglycosides (streptomycin), which bound to a single ribosomal site, and the first quinolone (nalidixic acid), which attached to only one of the four topoisomerase subunits. Newer agents in each class bind at multiple sites on their target, making mutation to resistance statistically improbable.

PBPs are altered transpeptidases

One of the most important examples of altered target involves the $\beta$-lactam family and the peptidoglycan transpeptidase penicillin-binding proteins (PBPs) on which they act. In widely divergent Gram-positive and Gram-negative species, changes in one or more of these proteins have been correlated with decreased susceptibility to multiple $\beta$-lactams. These alterations were initially detected as changes in electrophoretic migration of one or more PBPs using radiolabeled penicillin (hence the origin of the term PBP). These changes have now been traced to point mutations, substitutions of amino acid sequences, and even synthesis of a new enzyme.

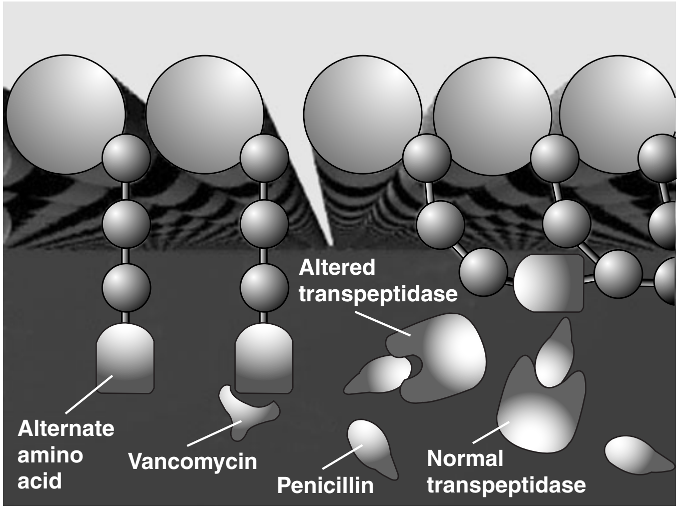



FIGURE 14–4
Altered target resistance. (For a diagrammatic representation of peptidoglycan synthesis, see Figure 13–1.) A normal transpeptidase is inactivated by penicillin, but penicillin no longer attaches to those with altered binding sites. The transpeptidase is still able to carry out its cross-linking function so the $\beta$-lactam is no longer effective. Here, vancomycin is no longer able to bind to its usual site, because another amino acid with a different shape has been substituted.

Because the altered binding is not absolute, decreases in susceptibility are incremental and often small. Wild-type pneumococci and gonococci are inhibited by 0.06 $\mu$g/mL of penicillin, while those with altered PBPs have MICs of 0.1 to 8.0 $\mu$g/mL. At the lower end, these MICs still appear to be within therapeutic range but are associated with treatment failures, even when dosage is increased. Altered PBPs generally affect all $\beta$-lactams. Although the exact MICs vary, a strain with a 10-fold decrease in susceptibility to penicillin has decreased susceptibility to cephalosporins to about the same degree.

Altered PBPs have reduced affinity for $\beta$-lactams

Penicillins and cephalosporins are affected to the same degree

PBP alterations are the prime reason for emergence of penicillin-resistant pneumococci and methicillin-resistant *Staphylococcus aureus* (MRSA). They are one of multiple mechanisms of resistance for a variety of other bacteria including enterococci, gonococci, *Haemophilus in uenzae*, and many other Gram-positive and Gram-negative species.

Pneumococci and MRSA have altered PBPs

Alteration of the target does not require mutation and can occur by the action of a new enzyme produced by the bacteria. Vancomycin-resistant enterococci have enzyme systems that substitute an amino acid in the terminal position of the peptidoglycan side chain (alanyl lysine for alanyl alanine). Vancomycin does not bind to the alternate amino acid, and these strains are resistant. Resistance to sulfonamides and trimethoprim occurs by acquisition of new enzymes with low affinity for these agents but still allows bacterial cells to carry out their respective functions in the folate synthesis pathway.

New enzymes can alter bacterial targets

Clindamycin resistance involves an enzyme that methylates ribosomal RNA, preventing attachment. This modification also confers resistance to erythromycin and other macrolides, because they share binding sites. Interestingly, induction with erythromycin leads to clindamycin resistance, although the reverse is unusual.

Mutation or acquisition of a new enzyme is possible

## *Enzymatic Inactivation (Fig 14–5)*

Enzymatic inactivation of the invading antimicrobic is the most powerful and robust of the resistance mechanisms. Literally hundreds of distinct enzymes produced by resistant bacteria may inactivate the antimicrobic in the cell, in the periplasmic space, or outside the cell. They may act on the antimicrobic molecule by disrupting its structure or by catalyzing a reaction that chemically modifies it.

Enzymes may disrupt or chemically modify antimicrobics

***$\beta$-Lactamases*** $\beta$-Lactamase is a general term referring to any one of hundreds of bacterial enzymes able to break open the $\beta$-lactam ring and inactivate various members of the $\beta$-lactam group. The first was discovered when penicillin-resistant strains of *S. aureus* emerged and were found to inactivate penicillin in vitro. The enzyme was called penicillinase, but with expansion of the $\beta$-lactam family and concomitant resistance, it has become clear that the situation is quite complex. Each $\beta$-lactamase is a distinct enzyme with its own physical characteristics and substrate profile. For example, the

Enzymes break open the $\beta$-lactam ring

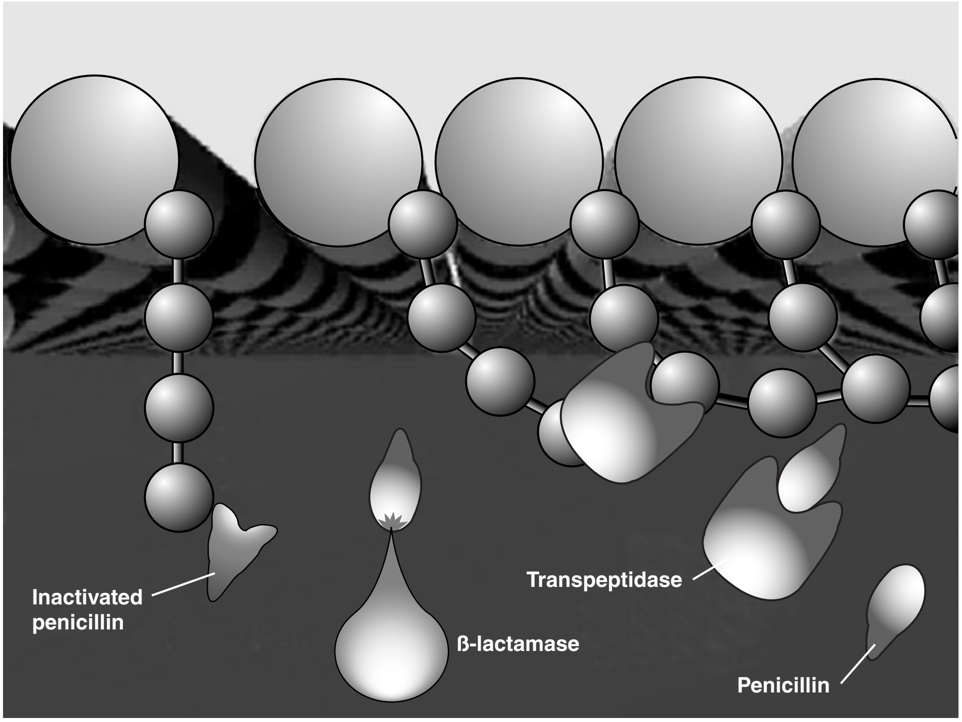



FIGURE 14–5
Enzymatic inactivation resistance. (For a diagrammatic representation of peptidoglycan synthesis, see Figure 13–1.) The bacterium is producing a $\beta$-lactamase enzyme, which destroys penicillin by breaking open the $\beta$-lactam ring. If the penicillin can reach the transpeptidase, it can still inactivate it; the more $\beta$-lactamase produced, the higher the level of resistance.

$\beta$-Lactamases have variable activity against $\beta$-lactam substrates

original staphylococcal penicillinase is also active against ampicillin but not against methicillin or any cephalosporin. $\beta$-Lactamases produced by *Escherichia coli* may add cephalosporinase activity but vary in their potency against individual first-, second-, third-, and fourth-generation cephalosporins. Some $\beta$-lactamases are bound by clavulanic acid, and others are not.

To keep track of the $\beta$-lactamase identifiers (eg, TEM-1, TEM-2, OXA, SVH), classification schemes have been created based on molecular structure, substrate profile, and inducibility (ie, whether enzymes are inducible or produced constitutively). A consideration of $\beta$-lactamase classification is beyond the scope of this book, but some discussion of the major types is useful. Gram-positive $\beta$-lactamases are exoenzymes with little activity against cephalosporins or the antistaphylococcal penicillins (methicillin, oxacillin). They are bound by $\beta$-lactamase inhibitors such as clavulanic acid. Gram-negative enzymes act in the periplasmic space and may have penicillinase and/or cephalosporinase activity. They may or may not be inhibited by clavulanic acid. Many of the Gram-negative $\beta$-lactamases are constitutively produced at very low levels but can be induced to high levels by exposure to a $\beta$-lactam agent. A newer class, called extended-spectrum $\beta$-lactamases (ESBLs) because their range includes multiple cephalosporins, is particularly worrisome. The laboratory detection of ESBLs is complex because they are inducible enzymes, and the conditions for induction may not be met in the susceptibility test.

May be exoenzymes or act in periplasmic space

ESBLs have broad activity against cephalosporins

Bacteria that produce $\beta$-lactamases typically demonstrate high-level resistance with MICs far outside the therapeutic range. Even weak $\beta$-lactamase producers are considered resistant because the outcome of susceptibility tests (and presumably infected sites) is strongly influenced by the number of bacteria present. Rapid direct tests for $\beta$-lactamase can provide this information in a few minutes.

Weak $\beta$-lactamase producers are still considered resistant

***Modifying Enzymes*** The most common cause of acquired bacterial resistance to aminoglycosides is through production of one or more of over 50 enzymes that acetylate, adenylate, or phosphorylate hydroxyl or amino groups on the aminoglycoside molecule. The modifications take place in the cytosol or in close association with the cytoplasmic membrane. The resistance conveyed by these actions is usually high level; the chemically modified aminoglycoside no longer binds to the ribosome. As with the $\beta$-lactamases, the aminoglycoside-modifying enzymes represent a large and diverse group of bacterial proteins, each with its characteristic properties and substrate profile. Inactivating enzymes have been described for a number of other antimicrobics. Most of these act by chemically modifying the antimicrobic molecule in a manner similar to the aminoglycoside-modifying enzymes. The most clinically significant enzymes convey resistance to erythromycin (esterase, phosphotransferase) and chloramphenicol (acetyltransferase).

Chemically modified aminoglycosides do not bind to ribosomes

## Genetics of Resistance

### *Intrinsic Resistance*

For any antimicrobic, there are bacterial species that are typically within its spectrum and those which are not. The resistance of the latter group is referred to as **intrinsic** or **chromosomal** to reflect its inherent nature. The resistant species have features such as permeability barriers, a lack of susceptibility of the cell wall, or ribosomal targets that make them inherently insusceptible. Some species constitutively produce low levels of inactivating enzymes, particularly the $\beta$-lactamases of Gram-negative bacteria. The chromosomal genes encoding these $\beta$-lactamases may be under repressor control and subject to induction by certain $\beta$-lactam antimicrobics. This leads to increased production of $\beta$-lactamase, which usually results in resistance not only to the inducer but other $\beta$-lactams to which the organism would otherwise be susceptible. Many of the ESBLs operate in this manner.

Permeability barriers or enzyme production may be intrinsic

Inducible enzymes may have broad spectrum

### *Acquired Resistance*

When an initially susceptible species develops resistance, such acquired resistance can be mutational or derived from another organism by the acquisition of new genes using one of the mechanisms of genetic exchange described in Chapter 4. Of these, conjugation and transposition are the most important and often work in tandem.

***Mutational Resistance*** Acquired resistance may occur when there is a crucial mutation in the target of the antimicrobic or in proteins related to access to the target (ie, permeability). Mutations in regulatory proteins can also lead to resistance. Mutations take place at a regular but low frequency and are expressed only if they are not associated with other effects that are disadvantageous to the bacterial cell. Mutational resistance can emerge in a single step or evolve slowly requiring multiple mutations before clinically significant resistance is achieved. Single-step mutational resistance is most likely when the antimicrobic binds to a single site on its target. Resistance can also emerge rapidly when it is related to gene regulation, such as mutational derepression of a chromosomally encoded cephalosporinase. A slow, progressive resistance evolving over years, even decades, is typical for $\beta$-lactam resistance related to altered PBPs.

Mutations in structural or regulatory genes can confer resistance

Mutations are usually low-frequency

***Plasmids and Conjugation*** The transfer of plasmids by conjugation was the first discovered mechanism for acquisition of new resistance genes, and it continues to be the most important. Resistance genes on plasmids (R plasmids) can determine resistance to one antimicrobic or to several that act by different mechanisms. After conjugation, the resistance genes may remain on a recircularized plasmid or less often become integrated into the chromosome by recombination. Of course, resistance is not the only concern of plasmids. A single cell may contain more than one distinct plasmid and/or multiple copies of the same plasmid. Although most resistance mechanisms have been linked to plasmids in one species or another, plasmid distribution among the bacterial pathogens is by no means uniform. The compatibility systems that maintain plasmids from one bacteria cell generation to the next are complex. Some species of bacteria are more likely than others

Plasmid conjugation allows multidrug resistance

Species may carry multiple or no plasmids to contain plasmids at all. For example, *Neisseria gonorrhoeae* typically has multiple plasmids, whereas closely related *Neisseria meningitidis* rarely has any.

Conjugation genes and host range enhance plasmid spread

Plasmids are most likely to be transferred to another strain if they are conjugative, that is, if the resistance plasmid also contains the genes mediating conjugation. Another factor in the spread of plasmids is their host range. Some plasmids can be transferred only to closely related strains; others can be transferred to a broad range of species in and beyond their own genus. A conjugative plasmid with a broad host range has great potential to spread any resistance genes it carries.

Transposons resistance genes move between chromosomes and plasmids

Transposition and conjugation combine for resistance spread

***Transposons and Transposition*** Transposons containing resistance genes can move from plasmid to plasmid or between plasmid and chromosome. Many of the resistance genes carried on plasmids are transposon insertions which can be carried along with the rest of the plasmid genome to another strain by conjugation. Once there, the transposon is free to remain in the original plasmid, insert in a new plasmid, insert in the chromosome, or any combination of these (Fig 14–6). Theoretically, plasmids can accomplish the same events by recombination, but the nature of the transposition process is such that it is much more likely to result in the transfer of an intact gene. Transposons also have a variable host range which in general is even broader than plasmids. Together, conjugation and transposition provide extremely efficient means for spreading resistance genes.

Transduction is limited by specificity of bacteriophages

Importance of transformation is unknown

***Other Genetic Mechanisms*** Although the transfer of resistance genes by transduction has been demonstrated in the laboratory, its association to clinically significant resistance has been uncommon. Transduction of imipenem resistance by wild-type bacteriophages carried by *P. aeruginosa* to other strains of the same bacteria is a recent example. Because of the high specificity of bacteriophages, transduction is typically limited to bacteria of the same species. Transformation is the most common way genes are manipulated in the laboratory, but detecting its occurrence in the clinical situation is particularly difficult. Plasmids are readily isolated and characterized, and transposons have flanking insertion sequences to flag their presence, but there is little to mark the uptake of naked DNA. Molecular epidemiologic studies suggest that the spread of PBP mutations in *Streptococcus pneumoniae* is due to transformation, and there may be many more examples awaiting discovery.

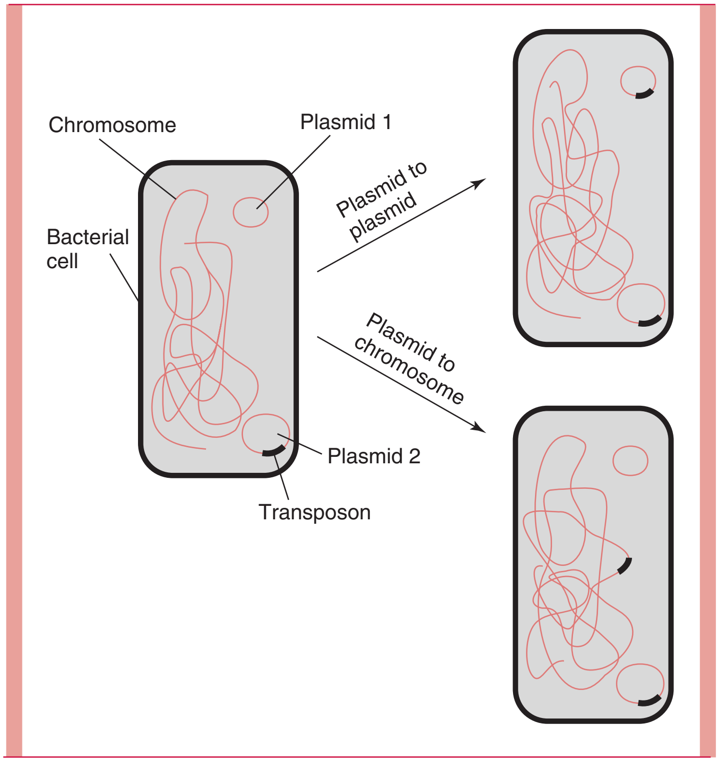



FIGURE 14–6
Plasmids and transposons. When passed to the next generation, transposons incorporated in plasmids may be inserted in another plasmid or in the chromosome.

## Epidemiology of Resistance

Clinical use is followed by resistance

The laws of evolution dictate that sooner or later microorganisms will develop resistance to any antimicrobic to which they are exposed. Since the start of the "antibiotic era," each new antimicrobic has tended to go through a remarkably similar sequence. When an agent is first introduced, its spectrum of activity seems almost completely predictable; some species are naturally resistant, and others are susceptible, with few exceptions. With clinical use, resistant strains of previously susceptible species begin to appear and become increasingly common.

Predominant susceptibility can turn to resistance

Resistance may emerge after decades of use

Some pathogens remain universally susceptible

In some situations, resistance develops rapidly; in other cases it takes years, even decades. For example, when penicillin was first introduced in 1944, all strains of *S. aureus* appeared to be fully susceptible to this antimicrobic. By 1950, less than one third of isolates remained susceptible. Currently, that figure has now declined below 15%. On the other hand, the discovery of *Haemophilus in uenzae* strains resistant to ampicillin did not occur until ampicillin had been used heavily for more than a decade. Penicillin was the primary treatment for pneumonia and meningitis caused by *S. pneumoniae* for 30 years before resistance emerged. Enteric Gram-negative rods rapidly developed resistance to antimicrobics such as ampicillin, cephalosporins, tetracycline, chloramphenicol, and aminoglycosides, with many strains becoming resistant to as many as 15 agents. Fortunately, these developments have not been universal. The spirochete of syphilis and the group A streptococcus have thus far retained their susceptibility to penicillin.

### *Origin of Resistant Strains*

Preexisting strains are selected by antimicrobial use

Resistant strains may exist prior to the introduction of an antimicrobic but at a frequency so small they are unlikely to be detected. For example, penicillinase-producing *S. aureus* have been found in culture collections that proceeded the development and use of this antibiotic. Under the selective pressure provided by use of any antimicrobic, preexisting resistant clones are likely to increase and, if they are virulent, spread.

Plasmid carriage may have survival value

The origin of plasmid-carried determinants of resistance remains somewhat obscure. Some may have played a role in nature by protecting the organism from antimicrobics produced by another organism or even for protection of the cell from its own antibiotic. Plasmids and transposons carrying resistance genes have little, if any, adverse influence on the capacity of most organisms to survive, infect, and spread.

### *Enhancement and Spread of Resistance*

Antimicrobial use creates an ecologic vacuum

Broad-spectrum effects are greatest

Plasmids amplify availability of resistance genes

The central factors involved in the increasing incidence of resistance are the selective effect of the use of antimicrobics, the spread of infection in human populations, and the ability of plasmids to cross species and even generic lines. Therapeutic or prophylactic use of antimicrobics, particularly those with a broad spectrum of activity, produces a relative ecologic vacuum in sites with a normal flora or on lesions prone to infection and allows resistant organisms to colonize or infect with less competition from others. Treatment with a single antimicrobic may select for strains that are also resistant to many other agents. Thus, chemotherapy can both enhance the opportunity for acquiring resistant strains from other sources and increase their numbers in the body. The amplifying effect of antimicrobial therapy on resistance is also apparent with the transfer of resistance plasmids to previously susceptible strains. This effect has been most clearly demonstrated in the lower intestinal tract, where the antimicrobic may reduce the flora and also produce an increased oxidation–reduction potential that favors plasmid transfer.

Benign *E. coli* can transfer multiple resistance genes to virulent *Shigella* in the intestine

As an example, consider a male patient harboring a strain of *E. coli* carrying a plasmid with genes encoding resistance to tetracycline, ampicillin, chloramphenicol, and the sulfonamides as a very small part of his facultative intestinal flora. He develops an infection with *Shigella dysenteriae* that is susceptible to all of these antimicrobics and is treated with tetracycline. Most of the normal flora and the *Shigella* are inhibited, but the resistant *E. coli* increases because its multiplication is not impeded and competition is removed. Plasmid transfer occurs between the resistant *E. coli* and some surviving

*Shigella,* which then multiply, causing a relapse of the disease with a strain that is now multiresistant. Any endemic or epidemic spread of dysentery from this patient to others will now be with the multiresistant *Shigella* strain, and its ability to infect will be enhanced if the recipient is on prophylaxis or therapy with any of the four antimicrobics to which it is resistant.

Antimicrobics in animal feeds increase the resistant population

Outbreaks have been traced from patients back to farms

The use of antimicrobics added to animal feeds for their growth-promoting effects represents a major source of resistant strains. Cattle or poultry that consume feed supplemented with antimicrobics rapidly develop a resistant enteric flora that spreads throughout the herd. Resistance is largely plasmid determined and has been shown capable of spreading to the flora of those living in close proximity to cattle-rearing farms. The links to human disease have been established, particularly for bacteria where these animals are the direct reservoir for human infection. For example, the techniques of molecular epidemiology have allowed the tracing of resistance plasmids involved in outbreaks of *Salmonella* gastroenteritis from the contaminated food back to the food processing plant and then to the originating farm. As a consequence, many countries have banned or controlled addition to animal feeds of antimicrobics that are useful for systemic therapy in humans. The United States has not yet taken any action because of opposition by business forces in the animal husbandry industry that fear lost profits.

### *Control of Resistance*

In the past, numerous examples in the literature showed that the extent of resistance in a hospital directly reflects the extent of usage of an antimicrobic, and that withdrawal or control can lead to rapid reduction of the incidence of resistance. Although this is more difficult to demonstrate in the community setting, experience and our understanding of the mechanisms and spread of resistance indicate that certain principles can help keep the problem under control:

1. Use antimicrobics conservatively and specifically in therapy.
2. Use an adequate dosage and duration of therapy to eliminate the infecting organism and reduce the risk of selecting resistant variants.
3. Select antimicrobics according to the proved or anticipated known susceptibility of the infecting strain whenever possible.
4. Use narrow-spectrum rather than broad-spectrum antimicrobics when the specific etiology of an infection is known, if possible.
5. Use antimicrobic combinations when they are known to prevent emergence of resistant mutants.
6. Use antimicrobics prophylactically only in situations in which it has been proven valuable and for the shortest possible time to avoid selection of a resistant flora.
7. Avoid environmental contamination with antimicrobics.
8. Rigidly apply careful, aseptic and handwashing procedures to help prevent spread of resistant organisms.
9. Use containment isolation procedures for patients infected with resistant organisms that pose a threat to others, and use protective precautions for those who are highly susceptible.
10. Epidemiologically monitor resistant organisms or resistance determinants in an institution and apply enhanced control measures if a problem develops.
11. Restrict the use of therapeutically valuable antimicrobics for nonmedical purposes.

## Selection and Administration of Antibacterial Antimicrobics

Antimicrobics are effective along with other treatments

This topic is largely beyond the scope of this book, but a few principles merit emphasis. Most bacterial infections are now potentially curable by chemotherapy alone or its use as an adjunct to surgical or other treatment. However, the plethora of antimicrobics available to physicians makes selection of the most appropriate agent(s) particularly challenging. Although the clinical indications for use vary widely, they usually fit into one of three categories: empiric, specific, or prophylactic.

## *Empiric Therapy*

The first decisions on selection of antimicrobic(s) are based on the physician's assessment of the probable microbial etiology of the patient's infection. The variables involved are the subject of much of this book and include the site of infection (eg, throat, lung, urine) and epidemiologic factors such as age, season, geography, and predisposing conditions. A mental list of probable etiologies must then be matched with their probable antimicrobial susceptibilities as shown in Table 13–1. Specific local "batting averages" for each antimicrobic against the common organisms are available from hospital laboratories and infection control committees. Many astute clinicians carry statistics concerning bacterial effectiveness in a pocket.

Probable etiology and susceptibility statistics guide initial selection

This process may be as simple as selecting penicillin to treat a patient with suspected group A streptococcal pharyngitis, or as complex as resorting to a cocktail of broad-spectrum antibacterial, antifungal, and antiviral agents to treat a febrile patient who has had a bone marrow transplant. In general, the risks of broad-spectrum treatment (superinfection, overgrowth) become more tolerable as the severity of the infection increases. When the risk of not "covering" an improbable pathogen is death, it is difficult to be selective. This treatment selection based on clinical criteria alone must be coupled with appropriated diagnostic steps (see Chapter 15) to determine the etiology, so the empiric therapy can be converted to specific therapy as quickly as possible.

Narrow versus broad spectrum is influenced by clinical severity

## *Specific Therapy*

Specific antimicrobial therapy is directed at the known agent of infection, usually a single species. It is unique to infectious diseases and is made possible by isolation and identification of the microorganism from the patient. In the case of bacterial diseases, it can even be made specific to the patient's own isolate by the use of antimicrobial susceptibility tests. The ideal goal of specific therapy is to attack the infecting organism and nothing else—to be the mythical "silver bullet." As the results of Gram smears, cultures, and susceptibility tests are gathered from the laboratory, unnecessary antimicrobics can be discontinued and the spectrum of therapy narrowed as much as possible. For example, a patient with suspect staphylococcal or streptococcal infection might be empirically started on a cephalosporin to cover both possibilities. The isolation of a *S. aureus* susceptible to a cephalosporin and oxacillin but resistant to penicillin requires reassessment of that regimen. Even though the cephalosporin is active, the oxacillin is the better choice, because its narrower spectrum carries less risk of complications for the patient and reduces the selective pressure for emergence of resistance.

Isolation of the causative agent allows narrowing of spectrum

Susceptibility tests provide final guide

In general, the best specific therapy is a single antimicrobic, but there are exceptions. Two or more antimicrobics acting by different mechanisms may be combined to reduce the possibility that mutations to resistance can be expressed. This is particularly true for chronic infections such as tuberculosis, in which the microbial load is high and the treatment period is long. For example, if a lesion contains $10^9$ organisms, and the frequency of resistant mutants is $10^{-6}$, the chance of relapse by selection of a resistant mutant is significant. Adding a second drug with the same mutation rate but a different mechanism requires a double mutant for expression of the resistance in the patient. Because the chance of this event is to $10^{-12}$, the addition of a second antimicrobic should prevent development of resistance during therapy.

Pharmacologic combinations reduce the chances that resistant mutants are expressed

Another indication for antimicrobial combinations is the desire to achieve a greatly enhanced biologic effect called **synergism.** For example, relatively low concentrations of a $\beta$-lactam and an aminoglycoside may be bactericidal for *Enterococcus faecalis* when combined, but neither agent is lethal at clinically achievable levels. This occurs because inhibition of cell wall synthesis by penicillin allows passage of the aminoglycoside to its ribosomal target in the cell. Unfortunately, combinations may also be **antagonistic**. This happens when the action of one antimicrobic partially prevents the second from expressing its activity. Examples include certain combinations of bacteriostatic antimicrobics with a $\beta$-lactam antimicrobic, such as penicillin. Penicillin exerts its bacterial effect only on dividing cells, and inhibition of growth by a bacteriostatic antimicrobic may prevent

Combinations may be synergistic

Combination of a bacteriostatic agent and a $\beta$-lactam may be antagonistic the lethal activity of penicillin. Although specific therapy is the ideal, it is not always possible. Any degree of uncertainty about the etiologic diagnosis will broaden the therapeutic coverage, and in some instances an etiologic diagnosis may not even be attempted. Empirical treatment of acute otitis media usually stands, because reaching the middle ear to culture the specific etiology is judged to carry more risk and discomfort for the patient.

### *Prophylaxis*

Prophylaxis risks enhancing spread

Administration during procedures is most effective

The use of antimicrobics to prevent infection is a tempting but potentially hazardous endeavor. The risk for the individual patient is infection with a different, more resistant organism. The risks for the population are in increasing the pressure for the selection and spread of resistance. After many years of experience, the indications for antimicrobial prophylaxis have now been narrowed to a limited number of situations in which antimicrobics have been shown to decrease transmission during a period of high risk. Prophylaxis can reduce the risk of endogenous infection associated with certain surgical and dental procedures if given during the procedure (a few hours at most). The transmission of highly infectious bacteria to close contacts can also be reduced by prophylaxis. This has been effective for some pathogens spread by the respiratory route, such as the etiologic agents of meningitis, whooping cough, and plague. One of the outstanding successes of antimicrobial prophylaxis is the reduction of group B streptococcal sepsis and meningitis in neonates. In this instance, prophylactic penicillin is administered during labor to mothers with demonstrated vaginal group B streptococcal colonization.

## ADDITIONAL READING

Bradford PA. Extended-spectrum $\beta$-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 2001;14:933–951. This article addresses the difficulty in detecting the most potent family of inactivating enzymes.

Fluit AC, Maarten Visser MR, Schmitz FJ. Molecular detection of antimicrobial resistance. *Clin Microbiol Rev* 2001;14:836–871. This review focuses on the new molecular detection of resistance genes, and it also includes concise summaries of resistance mechanisms.

Zinner SH, Wise R, Moellering RC Jr, eds. Maximizing antimicrobial efficacy/minimizing antimicrobial resistance: A paradigm for the new millennium. *Clin Infect Dis* 2001;33(Suppl 3):S107–S235. The proceedings of a symposium held at the American Academy of Arts and Sciences address the broad issues of resistance such as overuse and strategies to reduce resistance in the population at large.

CHAPTER 15

# Principles of Laboratory Diagnosis of Infectious Diseases

KENNETH J. RYAN AND C. GEORGE RAY

The diagnosis of a microbial infection begins with an assessment of clinical and epidemiologic features, leading to the formulation of a diagnostic hypothesis. Anatomic localization of the infection with the aid of physical and radiologic findings (for example, right lower lobe pneumonia, subphrenic abscess) is usually included. This clinical diagnosis suggests a number of possible etiologic agents based on knowledge of infectious syndromes and their courses (see Chapters 59 through 72). The specific cause is then established by the application of methods described in this chapter. A combination of science and art on the part of both the clinician and laboratory worker is required: The clinician must select the appropriate tests and specimens to be processed and, where appropriate, suggest the suspected etiologic agents to the laboratory. The laboratory worker must use those methods that will demonstrate the probable agents, and be prepared to explore other possibilities suggested by the clinical situation or findings of the laboratory examinations. The best results are obtained when communication between the clinic and laboratory is maximal.

The general approaches to laboratory diagnosis vary with different microorganisms and infectious diseases. However, the types of methods are usually some combination of direct microscopic examinations, culture, antigen detection, and antibody detection (serology). Newer approaches involving direct detection of genomic components are also important, although few have become practical enough for routine use. In this chapter, these principles will be considered, with emphasis on their application to the diagnosis of diseases caused by bacteria and viruses. Most of the approaches to be described can also be applied, with certain variations, to the diagnosis of diseases caused by fungi and parasites. All of these begin with some kind of specimen collected from the patient.

Microscopic, culture, antigen, and antibody detection are classic methods

Genomic approaches are being developed

## THE SPECIMEN

The primary connection between the clinical encounter and diagnostic laboratory is the specimen submitted for processing. If it is not appropriately chosen and/or collected, no degree of laboratory skill will rectify the error. Failure at the level of specimen collection is the most common reason for failure to establish an etiologic diagnosis, or worse, for suggesting a wrong diagnosis. In the case of bacterial infections, the primary problem lies in distinguishing resident or contaminating normal floral organisms from those causing the infection. The three specimen categories illustrated in Figure 15–1 and discussed below are covered more specifically in Chapters 59 to 72.

Quality of the specimen is crucial

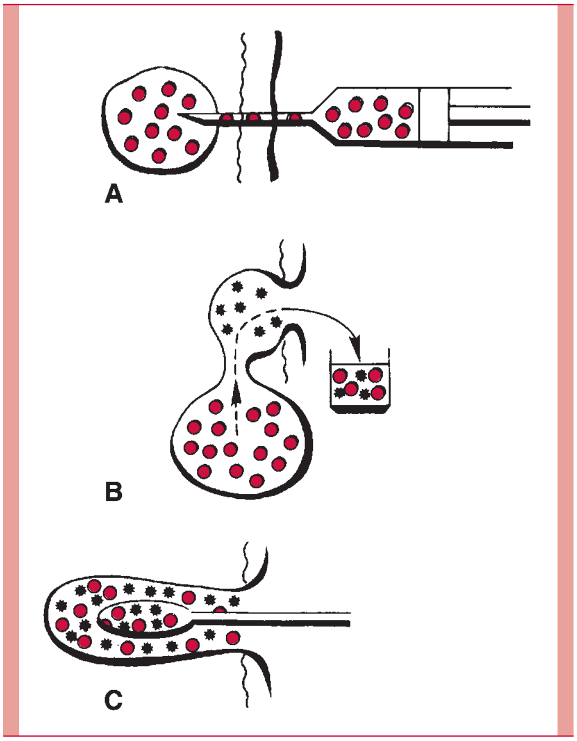



FIGURE 15–1
Specimens for the diagnosis of infection. **A.** Direct specimen. The pathogen (●) is localized in an otherwise sterile site, and a barrier such as the skin must be passed to sample it. This may be done surgically or by needle aspiration as shown. The specimen collected contains only the pathogen. Examples: deep abscess, cerebrospinal fluid. **B.** Indirect sample. The pathogen is localized as in A but must pass through a site containing normal flora (✱) in order to be collected. The specimen contains the pathogen but is contaminated with the nonpathogenic flora. The degree of contamination is often related to the skill with which the normal floral site was "bypassed" in specimen collection. Examples: expectorated sputum, voided urine. **C.** Sample from site with normal flora. The pathogen and nonpathogenic flora are mixed at the site of infection. Both are collected and the nonpathogen is either inhibited by the use of selective culture methods or discounted in interpretation of culture results. Examples: throat, stool.

## Direct Tissue or Fluid Samples

Direct samples give highest quality and risk

Direct specimens (Fig 15–1A) are collected from normally sterile tissues (lung, liver) and body fluids (cerebrospinal fluid, blood). The methods range from needle aspiration of an abscess to surgical biopsy. In general, such collections require the direct involvement of a physician and may carry some risk for the patient. The results are always useful, because positive findings are diagnostic and negative findings can exclude infection at the suspected site.

## Indirect Samples

Bypassing the normal flora requires extra effort

Results require interpretive evaluation of contamination

Indirect samples (Fig 15–1B) are specimens of inflammatory exudates (expectorated sputum, voided urine) that have passed through sites known to be colonized with normal flora. The site of origin is usually sterile in healthy persons; however, some assessment of the probability of contamination with normal flora during collection is necessary before these specimens can be reliably interpreted. This assessment requires knowledge of the potential contaminating flora (see Chapter 9) as well as the probable pathogens to be sought. Indirect samples are usually more convenient for both physician and patient, but carry a higher risk of misinterpretation. For some specimens, such as expectorated sputum, guidelines to assess specimen quality have been developed by correlation of clinical and microbiologic findings (see Chapter 64).

### Samples from Normal Flora Sites

Frequently the primary site of infection is in an area known to be colonized with many organisms (pharynx and large intestine) (Fig 15–1C). In such instances, examinations are selectively made for organisms known to cause infection that are not normally found at the infected site. For example, *Salmonella, Shigella,* and *Campylobacter* may be specifically sought in a stool specimen because they are known to cause diarrhea. It is neither practical nor relevant to describe the other stool flora.

Strict pathogens can be specifically sought

### Specimens for Viral Diagnosis

The selection of specimens for viral diagnosis is easier because there is essentially little normal viral flora to confuse interpretation. This allows selection guided by knowledge of which sites are most likely to yield the suspected etiologic agent. For example, enteroviruses are the most common viruses involved in acute infection of the central nervous system. Specimens that might be expected to yield these agents on culture include throat, stool, and cerebrospinal fluid.

Lack of normal viral flora simplifies interpretation

### Specimen Collection and Transport

The **sterile swab** is the most convenient and most commonly used tool for specimen collection; however, it provides the poorest conditions for survival and can only absorb a small volume of inflammatory exudate. The worst possible specimen is a dried-out swab; the best is a collection of 5 to 10 mL or more of the infected fluid or tissue. The volume is important because infecting organisms present in small numbers may not be detected in a small sample.

Swabs limit volume and survival

Specimens should be transported to the laboratory as soon after collection as possible, because some microorganisms survive only briefly outside the body. For example, unless special **transport media** are used, isolation rates of the organism that causes gonorrhea (*Neisseria gonorrhoeae*) are decreased when processing is delayed beyond a few minutes. Likewise, many respiratory viruses survive poorly outside the body. On the other hand, some bacteria survive well and may even multiply after the specimen is collected. The growth of enteric Gram-negative rods in specimens awaiting culture may in fact compromise specimen interpretation and interfere with the isolation of more fastidious organisms. Significant changes are associated with delays of more than 3 to 4 hours.

Viability may be lost if specimen is delayed

Various transport media have been developed to minimize the effects of the delay between specimen collection and laboratory processing. In general, they are buffered fluid or semisolid media containing minimal nutrients and are designed to prevent drying, maintain a neutral pH, and minimize bacterial growth. Other features may be required to meet special requirements, such as an oxygen-free atmosphere for obligate anaerobes.

Transport media stabilize conditions and prevent drying

## DIRECT EXAMINATION

Of the infectious agents discussed in this book, only some of the parasites are large enough to be seen with the naked eye. Bacteria can be seen clearly with the light microscope when appropriate methods are used; individual viruses can be seen only with the electron microscope, although aggregates of viral particles in cells (viral inclusions) may be seen by light microscopy. Various stains are used to visualize and differentiate microorganisms in smears and histologic sections.

All but some parasites require microscopy for visualization

### Light Microscopy

Direct examination of stained or unstained preparations by **light (bright field) microscopy** (Fig 15–2A) is particularly useful for detection of bacteria. Even the smallest bacteria (0.15 $\mu$m wide) can be visualized, although some require special lighting techniques. As the resolution limit of the light microscope is near 0.2 $\mu$m, the optics must be ideal if organisms are to be seen clearly by direct microscopy. These conditions may be achieved with a 100× oil immersion objective, a 5 to 10× eyepiece, and optimal lighting.

Bacteria are visible if optics are maximized

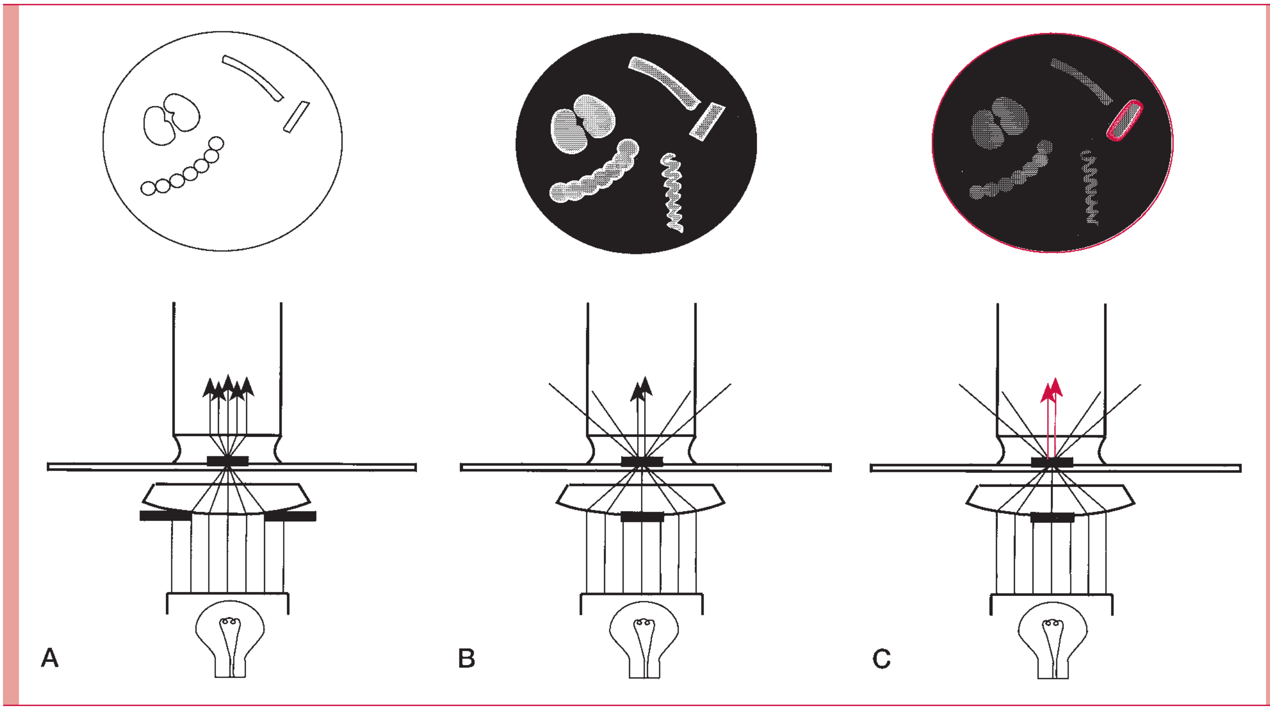



FIGURE 15–2
Bright-field, darkfield, and fluorescence microscopy. **A.** Bright-field illumination properly aligned. The purpose is to focus light directly on the preparation for optimal visualization against a bright background. **B.** In darkfield illumination, a black background is created by blocking the central light. Peripheral light is focused so that it will be collected by the objective only when it is reflected from the surfaces of particles (eg, bacteria). The microscopic field shows bright halos around some bacteria and reveals a spirochete too thin to be seen with bright-field illumination. **C.** Fluorescence microscopy is similar to darkfield microscopy, except the light source is ultraviolet and the organisms are stained with fluorescent compounds. The incident light generates light of a different wavelength, which is seen as a halo (colored in this illustration) around only the organism tagged with fluorescent compounds. For the most common fluorescent compound, the light is green.

Unstained bacteria are too transparent to see directly, although their presence can be indicated by the voids they create when suspended in particulate matter such as India ink.

Bacteria must be stained

Bacteria may be stained by a wide variety of dyes, including methylene blue, crystal violet, carbol-fuchsin (red), and safranin (red). The two most important methods, the Gram and acid-fast techniques, employ staining, decolorization, and counterstaining in a manner that helps to classify as well as stain the organism.

## *The Gram Stain*

Gram-positive bacteria retain purple iodine-dye complexes

Gram-negative bacteria do not retain complexes when decolorized

The differential staining procedure described in 1884 by the Danish physician Hans Christian Gram has proved one of the most useful in microbiology and medicine. The procedure (Fig 15–3) involves the application of a solution of iodine in potassium iodide to cells previously stained with an acridine dye such as crystal violet. This treatment produces a mordanting action in which purple insoluble complexes are formed with ribonuclear protein in the cell. The difference between Gram-positive and Gram-negative bacteria is in the permeability of the cell wall to these complexes on treatment with mixtures of acetone and alcohol solvents. This extracts the purple iodine–dye complexes from Gram-negative cells, whereas Gram-positive bacteria retain them. An intact cell wall is necessary for a positive reaction, and Gram-positive bacteria may fail to retain the stain if the organisms are old, dead, or damaged by antimicrobial agents. No similar conditions cause a Gram-negative organism to appear Gram positive. The stain is completed by the addition of red counterstain such as safranin, which is taken up by bacteria that have been

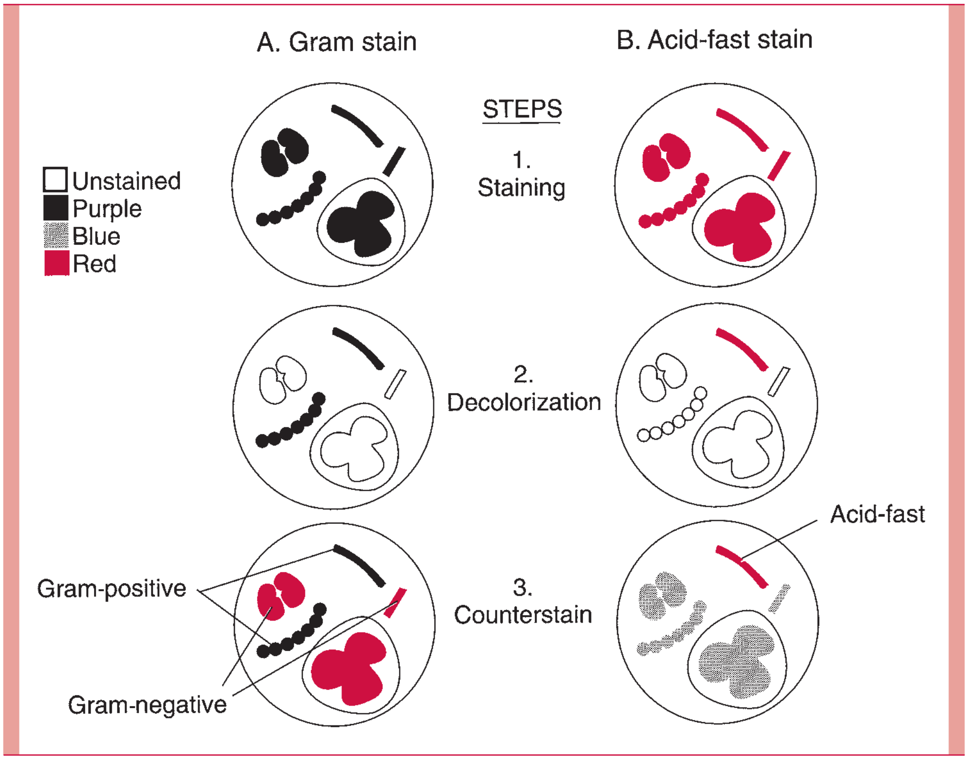



FIGURE 15–3 The Gram and acid-fast stains. Four bacteria and a PMN are shown at each stage. All are initially stained purple by the crystal violet and iodine of the Gram stain (A1) and red by the carbol fuchsin of the acid-fast stain (B1). Following decolorization, Gram-positive and acid-fast organisms retain their original stain. Others are unstained (A2, B2). The safranin of the Gram counterstain stains the Gram-negative bacteria and makes the background red (A3), and the methylene blue leaves a blue background for the contrasting red acid-fast bacillus (B3).

decolorized. Thus, cells stained purple are Gram positive, and those stained red are Gram negative. As indicated in Chapter 2, Gram positivity and negativity correspond to major structural differences in the cell wall.

When the Gram stain is applied to clinical specimens, the purple or red bacteria are seen against a Gram-negative (red) background of leukocytes, exudate, and debris. Retention of the purple dye in tissue or fluid elements, such as the nuclei of polymorphonuclear leukocytes, is an indication that the smear has been inadequately decolorized. In smears of uneven thickness, judgments on the Gram reaction can be made only in well-decolorized areas.

Properly decolorized background should be red

In many bacterial infections, the etiologic agents are readily seen on stained Gram smears of pus or fluids. This information, combined with the clinical findings, may guide the management of infection before culture results are available. Interpretation requires considerable experience and knowledge of probable causes, of their morphology and Gram reaction, and of any organisms normally present in health at the infected site.

Gram reaction plus morphology guide clinical decisions

## *The Acid-Fast Stain*

Acid fastness is a property of the mycobacteria (eg, *Mycobacterium tuberculosis*) and related organisms. Acid-fast organisms generally stain very poorly with dyes, including those used in the Gram stain. However, they can be stained by prolonged application of more concentrated dyes, and staining is facilitated by heat treatment. Their unique feature is that once stained, acid-fast bacteria resist decolorization by concentrations of mineral acids and ethanol that remove the same dyes from other bacteria. This combination of weak initial staining and strong retention once stained is probably related to the high lipid content of the mycobacterial cell wall. Acid-fast stains are completed with a counterstain to provide a contrasting background for viewing the stained bacteria (see Fig 15–3).

Acid-fast bacteria take stains poorly

Once stained retain it strongly

In the acid-fast procedure, the slide is flooded with carbol-fuchsin (red) and decolorized with hydrochloric acid in alcohol. When counterstained with methylene blue, acid-fast organisms appear red against a blue background. A variant is the **fluorochrome stain,** which uses a fluorescent dye, (auramine, or an auramine–rhodamine mixture) followed by decolorization with acid–alcohol. Acid-fast organisms retain the fluorescent stain, which allows their visualization by fluorescence microscopy.

There are multiple variants of the acid-fast stain

### *Darkfield and Fluorescence Microscopy*

Some bacteria, such as *Treponema pallidum*, the cause of syphilis, are too thin to be visualized with the usual bright-field illumination. They can be seen by use of the dark-field technique. With this method, a condenser focuses light diagonally on the specimen in such a way that only light reflected from particulate matter such as bacteria reaches the eyepiece (Fig 15–2B). The angles of incident and reflected light are such that the organisms are surrounded by a bright halo against a black background. This type of illumination is also used in other microscopic techniques, in which a high light contrast is desired, and for observation of fluorescence. Fluorescent compounds, when excited by incident light of one wavelength, emit light of a longer wavelength and thus a different color. When the fluorescent compound is conjugated with an antibody as a probe for detection of a specific antigen, the technique is called **immunofluorescence,** or fluorescent antibody microscopy. The appearance is the same as in darkfield microscopy except that the halo is the emitted color of the fluorescent compound (Fig 15–2C). For improved safety, most modern fluorescence microscopy systems direct the incident light through the objective from above (epifluorescence).

Darkfield creates a halo around organisms too thin to see by bright-field

Fluorescent stains convert darkfield to fluorescence microscopy

### *Electron Microscopy*

Electron microscopy demonstrates structures by transmission of an electron beam and has 10 to 1000 times the resolving power of light microscopic methods. For practical reasons its diagnostic application is limited to virology, where due to the resolution possible at high magnification it offers results not possible by any other method. Using negative staining techniques, direct examination of fluids and tissues from affected body sites enables visualization of viral particles. In some instances, electron microscopy has been the primary means of discovery of viruses that do not grow in the usual cell culture systems.

Viruses are visible only by electron microscopy

## CULTURE

Growth and identification of the infecting agent in vitro is usually the most sensitive and specific means of diagnosis and is thus the method most commonly used. Most bacteria and fungi can be grown in a variety of artificial media, but strict intracellular microorganisms (eg, *Chlamydia*, *Rickettsia*, and human and animal viruses) can be isolated only in cultures of living eukaryotic cells.

### Isolation and Identification of Bacteria

Almost all medically important bacteria can be cultivated outside the host in artificial culture media. A single bacterium placed in the proper culture conditions will multiply to quantities sufficient to be seen by the naked eye. Bacteriologic media are soup-like recipes prepared from digests of animal or vegetable protein supplemented with nutrients such as glucose, yeast extract, serum, or blood, to meet the metabolic requirements of the organism. Their chemical composition is complex, and their success depends on matching the nutritional requirements of most heterotrophic living things.

Bacteria grow in soup-like media

Growth in media prepared in the fluid state (broths) is apparent when bacterial numbers are sufficient to produce turbidity or macroscopic clumps. Turbidity results from reflection of transmitted light by the bacteria; depending on the size of the organism, more than $10^6$ bacteria per milliliter of broth are usually required. Most bacteria grow diffusely, but strictly aerobic bacteria may grow as a film on the surface of the broth, and other bacteria grow as a sediment. The addition of a gelling agent to a broth medium allows its preparation in solid form as plates in Petri dishes. The universal gelling agent for diagnostic bacteriology is **agar,** a polysaccharide extracted from certain types of seaweed. Agar has the convenient property of becoming liquid at about 95°C but not returning to the solid state as a gel until cooled to less than 50°C. This allows the addition of a heat-labile substance, such as blood, to the medium before it sets. At the temperatures used in the diagnostic laboratory (37°C or lower), broth–agar exists as a smooth, solid, nutrient gel. This medium, usually termed "agar," may be qualified with a description of any supplement (eg, blood agar).

Large numbers of bacteria in broth produce turbidity

Agar is a convenient gelling agent for solid media

Separation of bacteria may be accomplished by using a sterile wire loop to spread a small sample over the surface of an agar plate in a structured pattern called **plate streaking.** Bacteria that are well separated from others grow as isolated colonies, often reaching 2 to 3 mm in diameter after overnight incubation. For diagnostic work, growth of bacteria on solid media has advantages over the use of broth cultures. It allows isolation of bacteria in pure culture (Fig 15–4), because a colony well separated from others can be assumed to arise from a single organism or an organism cluster (colony-forming unit). Colonies vary greatly in size, shape, texture, color, and other features. For example, colonies of organisms possessing large polysaccharide capsules are usually mucoid; those of organisms that fail to separate after division are frequently granular. Colonies from different species or genera often differ substantially, whereas those derived from the same strain are usually consistent. Differences in **colonial morphology** are very useful for separating bacteria in mixtures and as clues to their identity. Some examples of colonial morphology are shown in Figure 15–5.

Bacteria may be separated in isolated colonies on agar plates

Colonies may have consistent and characteristic features

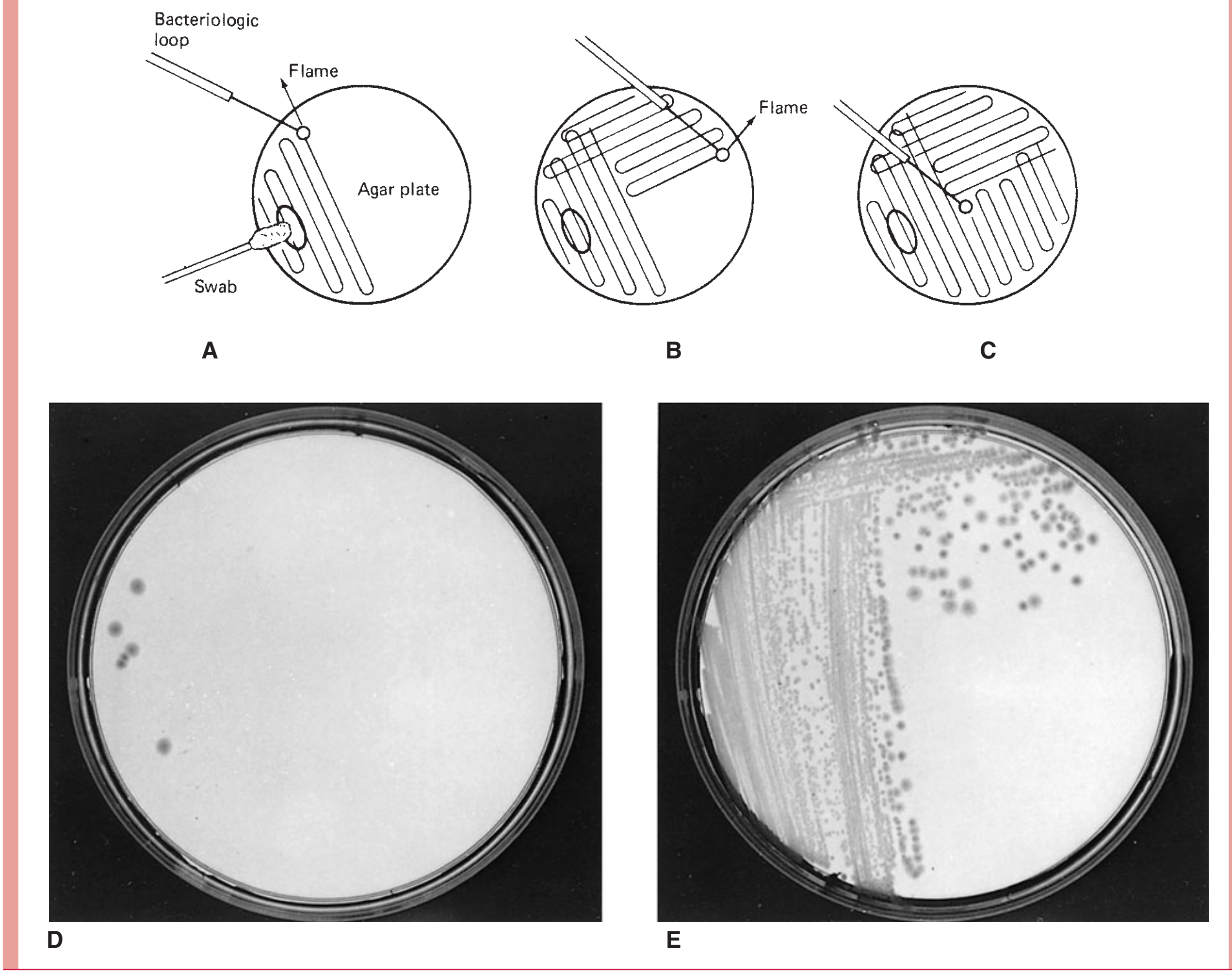



FIGURE 15–4
Bacteriologic plate streaking. Plate streaking is essentially a dilution procedure. **A.** The specimen is placed on the plate with a swab, loop, or pipette, and evenly spread over approximately one fourth of the plate surface with a sterilized bacteriologic loop. **B.** The loop is flamed to remove residual bacteria. A secondary streak is made, overlapping the primary streak initially but finishing independently. **C.** The process is repeated in a tertiary streak. **D.** and **E.** Two plates streaked in a similar manner. **D.** Only a few bacteria grew. **E.** A large number of bacteria grew. However, isolated colonies were produced for further study in each case.

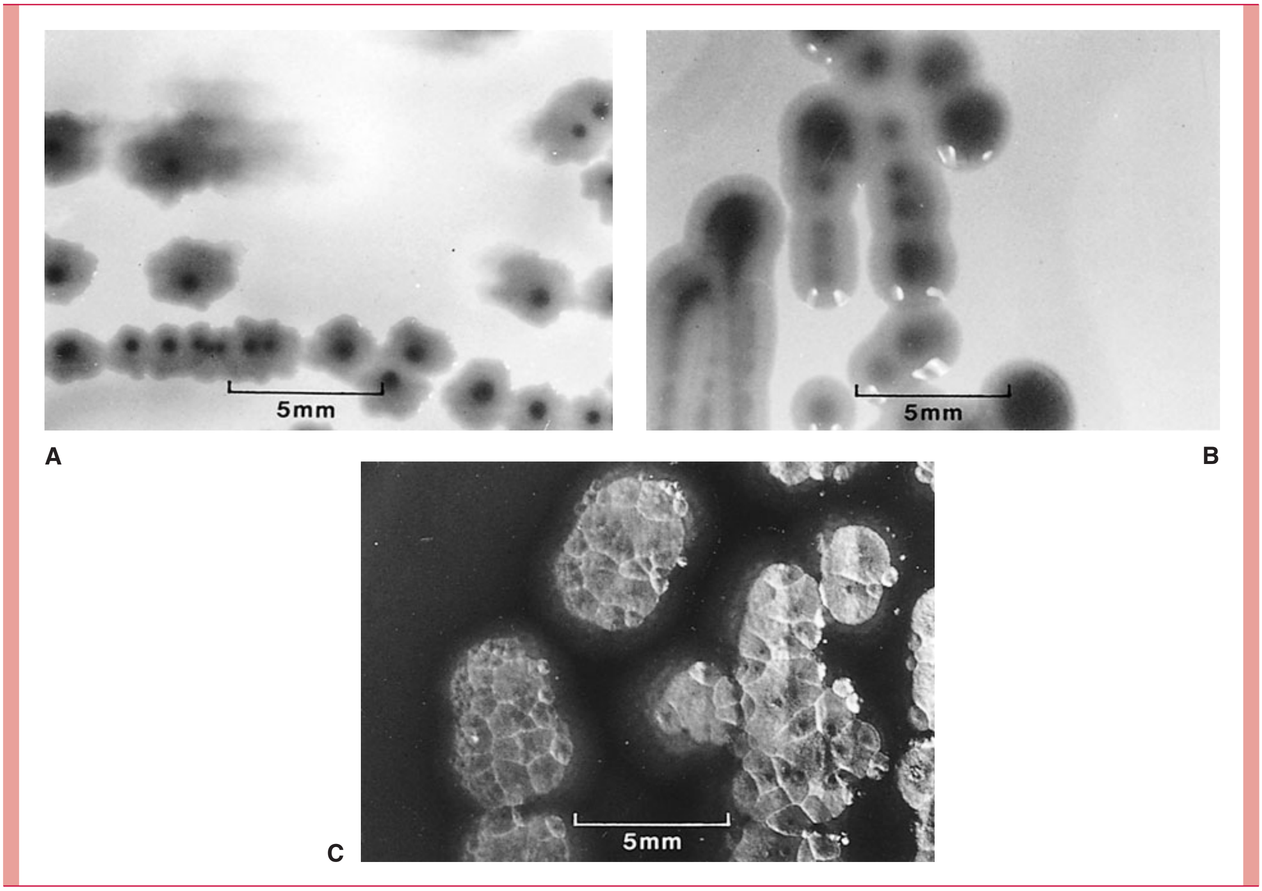



FIGURE 15–5
Bacterial colonial morphology. The colonies formed on agar plates by three different Gram-negative bacilli are shown at the same magnification. Each is typical for its species but variations are common. **A.** *Escherichia coli* colonies are flat with an irregular scalloped edge. **B.** *Klebsiella pneumoniae* colonies with a smooth entire edge and a raised glistening surface. **C.** *Pseudomonas aeruginosa* colonies with an irregular reflective surface, suggesting hammered metal.

Optical, chemical, and electrical methods can detect growth

New methods that do not depend on visual changes in the growth medium or colony formation are also used to detect bacterial growth in culture. These techniques include optical, chemical, and electrical changes in the medium, produced by the growing numbers of bacterial cells or their metabolic products. Many of these methods are more sensitive than classical techniques and thus can detect growth hours, or even days, earlier than classical methods. Some have also been engineered for instrumentation and automation. For example, one fully automated system that detects bacterial metabolism fluorometrically can complete a bacterial identification and antimicrobial susceptibility test in 2 to 4 hours.

## *Bacteriologic Media*

Over the past 100 years, countless media have been developed by bacteriologists to aid in the isolation and identification of medically important bacteria. Only a few have found their way into routine use in clinical laboratories. These may be classified as nutrient, selective, or indicator media.

Media are prepared from animal or plant products

***Nutrient Media*** The nutrient component of a medium is designed to satisfy the growth requirements of bacteria to permit isolation and propagation. For medical purposes, the ideal medium would allow rapid growth of all bacteria. No such medium exists; however, several suffice for good growth of most medically important bacteria. These media are prepared with enzymatic or acid digests of animal or plant products such as muscle, milk, or beans.

The digest reduces the native protein to a mixture of polypeptides and amino acids that also includes trace metals, coenzymes, and various undefined growth factors. For example, one common broth contains a pancreatic digest of casein (milk curd) and a papaic digest of soybean meal. To this nutrient base, salts, vitamins, or body fluids such as serum may be added to provide pathogens with the conditions needed for optimum growth.

***Selective Media*** Selective media are used when specific pathogenic organisms are sought in sites with an extensive normal flora (eg, *N. gonorrhoeae* in specimens from the uterine cervix or rectum). In these cases, other bacteria may overgrow the suspected etiologic species in simple nutrient media, either because the pathogen grows more slowly or because it is present in much smaller numbers. Selective media usually contain dyes, other chemical additives, or antimicrobics at concentrations designed to inhibit contaminating flora but not the suspected pathogen.

Unwanted organisms are inhibited with chemicals or antimicrobics

***Indicator Media*** Indicator media contain substances designed to demonstrate biochemical or other features characteristic of specific pathogens or organism groups. The addition to the medium of one or more carbohydrates and a **pH indicator** is frequently used. A color change in a colony indicates the presence of acid products and thus of fermentation or oxidation of the carbohydrate by the organism. Other indicator media may enhance the production of a **pigment** or other changes useful for early recognition of certain bacteria. The addition of red blood cells (RBCs) to plates allows the **hemolysis** produced by some organisms to be used as a differential feature (see Chapter 16). In practice, nutrient, selective, and indicator properties are often combined to various degrees in the same medium. It is possible to include an indicator system in a highly nutrient medium and also make it selective by adding appropriate antimicrobics. Some examples of culture media commonly used in diagnostic bacteriology are listed in Appendix 15–1, and more details of their constitution and application are provided in Appendix 15–2.

Metabolic properties of bacteria are demonstrated by substrate and indicator systems

## *Atmospheric Conditions*

***Aerobic*** Once inoculated, cultures of most aerobic bacteria are placed in an incubator with temperature maintained at 35 to 37°C. Slightly higher or lower temperatures are used occasionally to selectively favor a certain organism or organism group. Most bacteria that are not obligate anaerobes will grow in air; however, $CO_2$ is required by some and enhances the growth of others. Incubators that maintain a 2 to 5% concentration of $CO_2$ in air are frequently used for primary isolation, because this level is not harmful to any bacteria and improves isolation of some. A simpler method is the candle jar, in which a lighted candle is allowed to burn to extinction in a sealed jar containing plates. This method adds 1 to 2% $CO_2$ to the atmosphere.

Incubation temperature and atmosphere vary with organism

***Anaerobic*** Strictly anaerobic bacteria will not grow under the conditions described previously, and many will die if exposed to atmospheric oxygen or high oxidation–reduction potentials. Most medically important anaerobes will grow in the depths of liquid or semisolid media containing any of a variety of **reducing agents,** such as cysteine, thioglycollate, ascorbic acid, or even iron filings. An anaerobic environment for incubation of plates can be achieved by replacing air with a gas mixture containing hydrogen, $CO_2$, and nitrogen and allowing the hydrogen to react with residual oxygen on a catalyst to form water. A convenient commercial system accomplishes this chemically in a packet to which water is added before the jar is sealed. Specimens suspected to contain significant anaerobes should be processed under conditions designed to minimize exposure to atmospheric oxygen at all stages.

Anaerobes require reducing conditions and protection from oxygen

## *Clinical Bacteriology Systems*

Routine laboratory systems for processing specimens from various sites are needed because no single medium or atmosphere is ideal for all bacteria. Combinations of broth and solid-plated media and aerobic, $CO_2$, and anaerobic incubation must be matched to

Routine systems are designed to detect the most common organisms

TABLE 15–1

**Routine Use of Gram Smear and Isolation Systems for Selected Clinical Specimens[a]**

| | Specimen | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Medium (Incubation) | Blood | Cerebrospinal Fluid | Wound, Pus | Genital, Cervix | Throat | Sputum | Urine | Stool |
| Gram smear | | × | × | × | | × | × | |
| Soybean–casein digest broth ($CO_2$) | × | × | × | | | | | |
| Selenite F broth (air) | | | | | | | | × |
| Blood agar ($CO_2$) | | × | × | × | | × | × | |
| Blood agar (anaerobic) | | | × | | ×[b] | | | |
| MacConkey agar (air) | | | × | × | | × | × | × |
| Chocolate agar ($CO_2$) | | × | × | × | | × | | |
| Martin–Lewis agar ($CO_2$) | | | | × | | | | |
| Hektoen agar (air) | | | | | | | | × |
| *Campylobacter* agar ($CO_2$, 42°C)[c] | | | | | | | | × |

[a] The added sensitivty of a nutrient broth is used only when contamination by normal flora is unlikely. Exact media and isolation systems may vary between laboratories.
[b] Anaerobic incubation used to enhance hemolysis by $\beta$-hemolytic streptococci.
[c] Incubation in a reduced oxygen atmosphere.

the organisms expected at any particular site or clinical circumstance. Examples of such routines are shown in Table 15–1. In general, it is not practical to routinely include specialized media for isolation of rare organisms such as *Corynebacterium diphtheriae.* For detection of these and other uncommon organisms, the laboratory must be specifically informed of their possible presence by the physician. Appropriate media and special procedures can then be included.

## *Bacterial Identification*

Extent of identification is linked to medical relevance

Once growth is detected in any medium, the process of identification begins. Identification involves the use of methods to obtain pure cultures from single colonies, followed by tests designed to characterize and identify the isolate. The exact tests and their sequences vary with different groups of organisms, and the taxonomic level (genus, species, subspecies, and so on) of identification needed varies according to the medical usefulness of the information. In some cases, only a general description or the exclusion of particular organisms is important. For example, a report of "mixed oral flora" in a sputum specimen or "no *N. gonorrhoeae*" in a cervical specimen may provide all of the information needed.

### *Features Used to Classify Bacteria*

Growth under various conditions has differential value

**Cultural Characteristics** Cultural characteristics include the demonstration of properties such as unique nutritional requirements, pigment production, and the ability to grow in the presence of certain substances (sodium chloride, bile) or on certain media (MacConkey, nutrient agar). Demonstration of the ability to grow at a particular temperature or to cause hemolysis on blood agar plates is also used.

Biochemical reactions analyzed by tables and computers give identification probability

**Biochemical Characteristics** The ability to attack various substrates or to produce particular metabolic products has broad application to the identification of bacteria. The most common properties examined are listed in Appendix 15–3. Biochemical and cultural tests for bacterial identification are analyzed by reference to tables that show the reaction patterns characteristic for individual species. In fact, advances in computer analysis have now been applied to identification of many bacterial and fungal groups. These systems use the same biochemical principles along with computerized databases to determine the most probable identification from the observed test pattern.

**Toxin Production and Pathogenicity** Direct evidence of virulence in laboratory animals is rarely needed to confirm a clinical diagnosis. In some diseases caused by production of a specific toxin, the toxin may be detected in vitro through cell cultures or immunologic methods. Neutralization of the toxic effect with specific antitoxin is the usual approach to identify the toxin.

Detection of specific toxin may define disease

**Antigenic Structure** As discussed in Chapter 2, bacteria possess many antigens, such as capsular polysaccharides, flagellar proteins, and several cell wall components. Serology involves the use of antibodies of known specificity to detect antigens present on whole bacteria or free in bacterial extracts (soluble antigens). The methods used for demonstrating antigen–antibody reactions are discussed in a later section.

Antigenic structures of organism demonstrated with antisera

**Genomic Structure** Nucleic acid sequence relatedness as determined by homology comparisons have become a primary determinant of taxonomic decisions. They are discussed later in the section on nucleic acid methods.

## Isolation and Identification of Viruses

### *Cell and Organ Culture*

Living cell cultures that can support their replication are the primary means of isolating pathogenic viruses. The cells are derived from a tissue source by outgrowth of cells from a tissue fragment (explant) or by dispersal with proteolytic agents such as trypsin. They are allowed to grow in nutrient media on a glass or plastic surface until a confluent layer one cell thick (monolayer) is achieved. In some circumstances, a tissue fragment with a specialized function (eg, fetal trachea with ciliated epithelial cells) is cultivated in vitro and used for viral detection. This procedure is known as organ culture.

Cell cultures derived from human or animal tissues are used to isolate viruses

Three basic types of cell culture monolayers are used in diagnostic virology. The **primary cell culture,** in which all cells have a normal chromosome count (diploid), is derived from the initial growth of cells from a tissue source. Redispersal and regrowth produces a **secondary cell culture,** which usually retains characteristics similar to those of the primary culture (diploid chromosome count and virus susceptibility). Monkey and human embryonic kidney cell cultures are examples of commonly used primary and secondary cell cultures.

Monkey kidney is used in primary and secondary culture

Further dispersal and regrowth of secondary cell cultures usually leads to one of two outcomes: the cells eventually die, or they undergo spontaneous transformation, in which the growth characteristics change, the chromosome count varies (haploid or heteroploid), and the susceptibility to virus infection differs from that of the original. These cell cultures have characteristics of "immortality"; that is, they can be redispersed and regrown many times (serial cell culture passage). They can also be derived from cancerous tissue cells or produced by exposure to mutagenic agents in vitro. Such cultures are commonly called **cell lines.** A common cell line in diagnostic use is the *Hep*-2, derived from a human epithelial carcinoma. A third type of culture is often termed a **cell strain.** This culture consists of diploid cells, commonly fibroblastic, that can be redispersed and regrown a finite number of times; usually 30 to 40 cell culture passages can be made before the strain dies out or spontaneously transforms. Human embryonic tonsil and lung fibroblasts are common cell strains in routine diagnostic use.

Primary cultures either die out or transform

Cell strains regrow a limited number of times

Cells from cancerous tissue may grow continuously

### *Detection of Viral Growth*

Viral growth in susceptible cell cultures can be detected in several ways. The most common effect is seen with lytic or cytopathic viruses; as they replicate in cells, they produce alterations in cellular morphology (or cell death) that can be observed directly by light microscopy under low magnification (30× or 100×). This **cytopathic effect (CPE)**

Viral CPE is due to morphologic changes or cell death

CPE is characteristic for some viruses

varies with different viruses in different cell cultures. For example, enteroviruses often produce cell rounding, pleomorphism, and eventual cell death in various culture systems, whereas measles and respiratory syncytial viruses cause fusion of cells to produce multinucleated giant cells (syncytia). The microscopic appearance of some normal cell cultures and the CPE produced in them by different viruses are illustrated in Figure 15–6.

Hemadsorption or interference marks cells that may not show CPE

Other viruses may be detected in cell culture by their ability to produce **hemagglutinins.** These hemagglutinins may be present on the infected cell membranes, as well as in the culture media, as a result of release of free, hemagglutinating virions from the cells. Addition of erythrocytes to the infected cell culture results in their adherence to the cell surfaces, a phenomenon known as **hemadsorption.** Another method of viral detection in cell culture is by **interference.** In this situation, the virus that infects the susceptible cell culture produces no CPE or hemagglutinin, but can be detected by "challenging" the cell culture with a different virus that normally produces a characteristic CPE. The second, or challenge, virus fails to infect the cell culture because of interference by the first virus, which is thus detected. This method is obviously cumbersome, but has been applied to the detection of rubella virus in certain cell cultures.

EBV and HIV antigens are expressed on lymphocytes

Immunologic or genomic probes detect incomplete viruses

For some agents, such as Epstein–Barr virus (EBV) or human immunodeficiency virus (HIV), even more novel approaches may be applied. Both EBV and HIV can replicate in vitro in suspension cultures of normal human lymphocytes such as those derived from neonatal cord blood. Their presence may be determined in several ways; for example, EBV-infected B lymphocytes and HIV-infected T lymphocytes will express virus-specified antigens and viral DNA or RNA, which can be detected with immunologic or genomic probes. In addition, HIV reverse transcriptase can be detected in cell culture by specific assay methods. Immunologic and nucleic acid probes (see below) can also be used to detect virus in clinical specimens or in situations where only incomplete, noninfective virus replication has occurred in vivo or in vitro. An example is the use of in situ cytohybridization, whereby specific labeled nucleic acid probes are used to detect and localize papillomavirus genomes in tissues where neither infectious virus nor its antigens can be detected.

### *In Vivo Isolation Methods*

Embryonated eggs and animals are required for isolation of some viruses

In vivo methods for isolation are also sometimes necessary. The embryonated hen's egg is still used for the initial isolation and propagation of influenza A virus. Virus-containing material is inoculated on the appropriate egg membrane, and the egg is incubated to permit viral replication and recognition. Animal inoculation is still used for detecting some viruses. The usual animal host for viral isolation is the mouse; suckling mice in the first 48 hours of life are especially susceptible to many viruses. Evidence for viral replication is based on the development of illness, manifested by such signs as paralysis, convulsions, poor feeding, or death. The nature of the infecting virus can be further elucidated by histologic and immunofluorescent examination of tissues or by detection of specific antibody responses. Many arboviruses and rabies virus are detected in this system.

Specimen preparation is required

Time to detection varies from days to weeks

Viral isolation from a suspect case involves a number of steps. First, the viruses believed most likely to be involved in the illness are considered, and appropriate specimens are collected. Centrifugation or filtration and addition of antimicrobics are frequently required with respiratory or fecal specimens to remove organic matter, cellular debris, bacteria, and fungi, which can interfere with viral isolation. The specimens are then inoculated into the appropriate cell culture systems. The time between inoculation and initial detection of viral effects varies; however, for most viruses positive cultures are usually apparent within 5 days of collection. With proper collection methods and application of the diagnostic tools discussed later, many infections can even be detected within hours. On the other hand, some viruses may require culture for a month or more before they can be detected.

### *Viral Identification*

Nature of CPE and cell cultures affected may suggest virus

On isolation, a virus can usually be tentatively identified to the family or genus level by its cultural characteristics (eg, type of CPE produced). Confirmation and further identification may require enhancement of viral growth to produce adequate quantities for testing. This

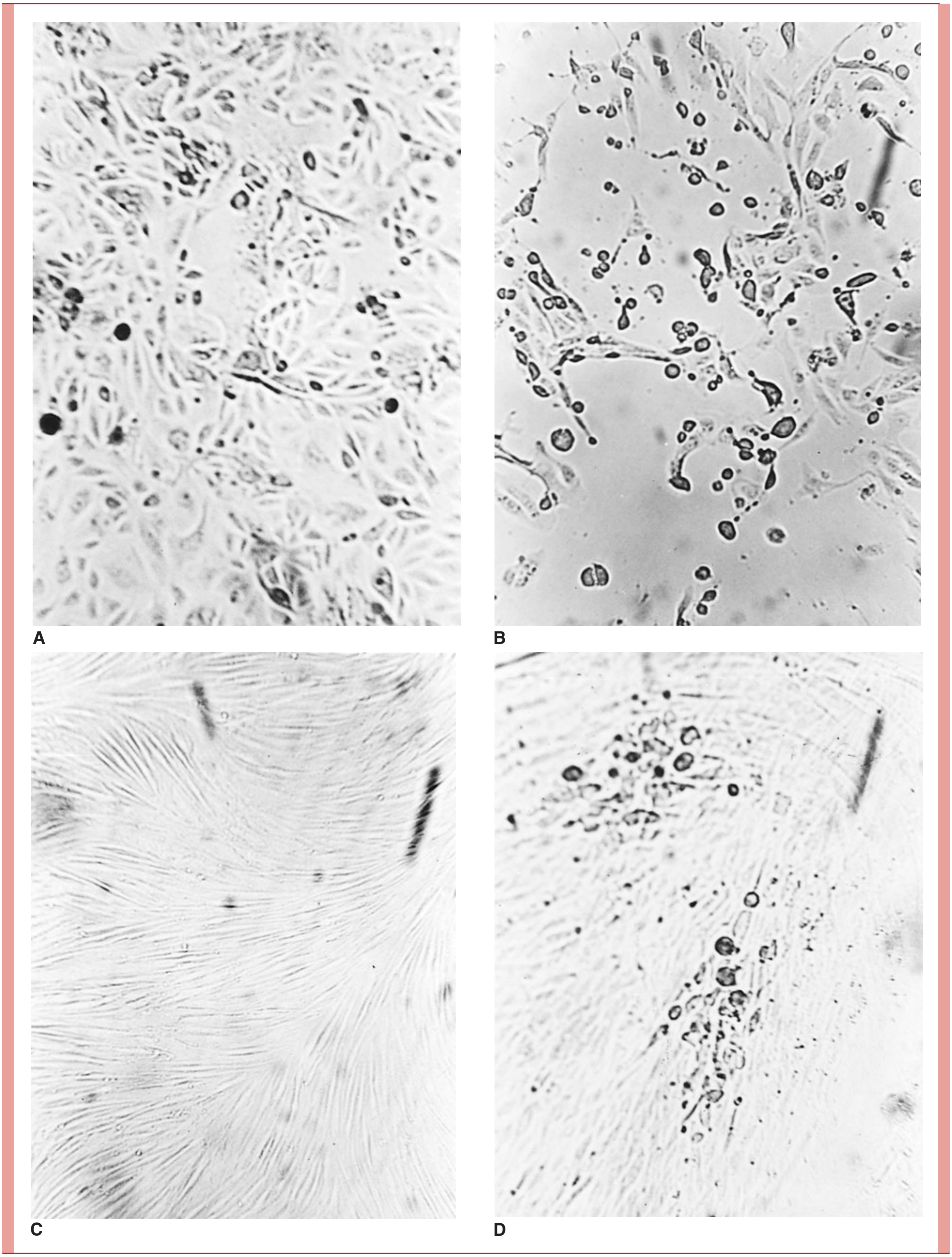



FIGURE 15–6

**A.** Normal monkey kidney cell culture monolayer. **B.** Enterovirus cytopathic effect in a monkey kidney cell monolayer. Note cell lysis and monolayer destruction. **C.** Normal human diploid fibroblast cell monolayer. **D.** Cytomegalovirus cytopathic effect in human diploid cell monolayer. Note rounded, swollen cells in a focal area. (A–D × 40.)

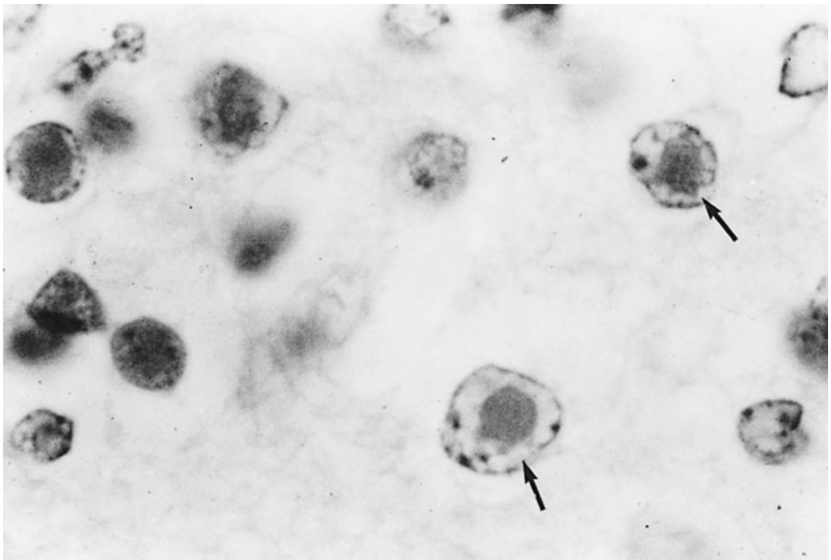


FIGURE 15–7
Brain biopsy from a patient with herpes simplex encephalitis. Arrows indicate infected neuronal nuclei with marginated chromatin and typical intranuclear inclusions. The cytoplasmic membranes are not clearly seen in this preparation (hematoxylin–eosin stain; × 400).

result may be achieved by inoculation of the original isolate into fresh culture systems (viral passage) to amplify replication of the virus, as well as improve its adaptation to growth in the in vitro system.

Neutralization of biologic effect with specific antisera confirms identification

***Neutralization and Serologic Detection*** Of the several ways to identify the isolate, the most common is to neutralize its infectivity by mixing it with specific antibody to known viruses before inoculation into cultures. The inhibition of the expected viral effects on the cell culture such as CPE or hemagglutination is then evidence for that virus. As in bacteriology, demonstration of specific viral antigens is a useful way to identify many agents. Immunofluorescence and enzyme immunoassay (EIA) are the most common methods.

Inclusions and giant cells suggest viruses

***Cytology and Histology*** In some instances, viruses will produce specific cytologic changes in infected host tissues that aid in diagnosis. Examples include specific intranuclear inclusions (herpes, Fig 15–7); cytoplasmic inclusions; and cell fusion, which results in multinucleated epithelial giant cells (chickenpox, Fig 15–8). Although such findings are useful when seen, their overall diagnostic sensitivity and specificity are usually considerably less than those of the other methods discussed.

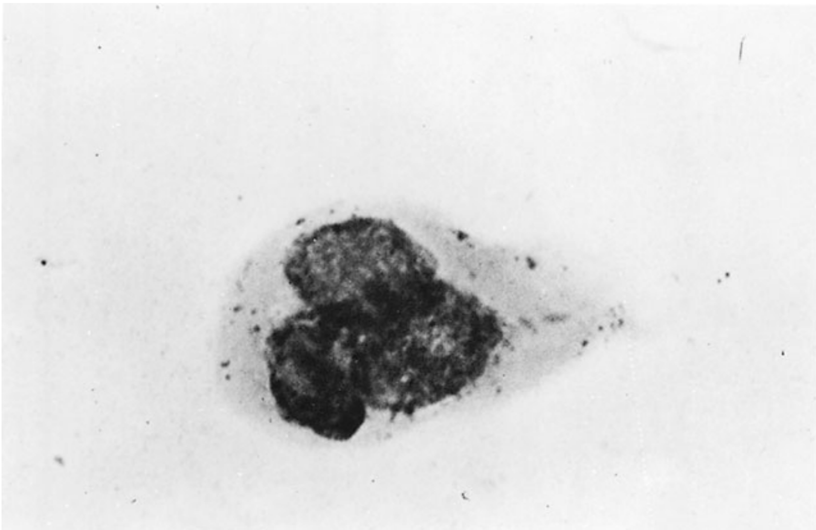


FIGURE 15–8
Multinucleated epithelial cells from a vesicle scraping of a patient with chickenpox. Cell fusion of this type can be seen with both varicella-zoster and herpes simplex infections (Wright's stain; × 400).

***Electron Microscopy*** When virions are present in sufficient numbers, they may be further characterized by specific agglutination of viral particles on mixture with type-specific antiserum. This technique, immune electron microscopy, can be used to identify viral antigens specifically or to detect antibody in serum using viral particles of known antigenicity.

Immune electron microscopy shows agglutinated viral particles

Some viruses (eg, human rotaviruses, hepatitis A and B viruses) grow poorly or not at all in the laboratory culture systems currently available. However, they can be efficiently detected by immunologic or molecular methods, to be described later in this chapter.

Not all viruses grow in culture

## IMMUNOLOGIC SYSTEMS

Diagnostic microbiology makes great use of the specificity of the binding between antigen and antibody. Antisera of known specificity are used to detect their homologous antigen in cultures, or more recently, directly in body fluids. Conversely, known antigen preparations are used to detect circulating antibodies as evidence of a current or previous infection with that agent. Many methods are in use to demonstrate the antigen–antibody binding. The greatly improved specificity of **monoclonal antibodies** has had a major impact on the quality of methods where they have been applied. Before discussing their application to diagnosis, the principles involved in the most important methods will now be discussed.

Antisera detect viral antigens

Viral antigens detect immune response

### Methods for Detecting an Antigen–Antibody Reaction

#### *Precipitation*

When antigen and antibody combine in the proper proportions, a visible precipitate is formed (Fig 15–9A). Optimum antigen–antibody ratios can be produced by allowing one to diffuse into the other, most commonly through an agar matrix **(immunodiffusion).**

Both the speed and the sensitivity of immunodiffusion are improved by CIE

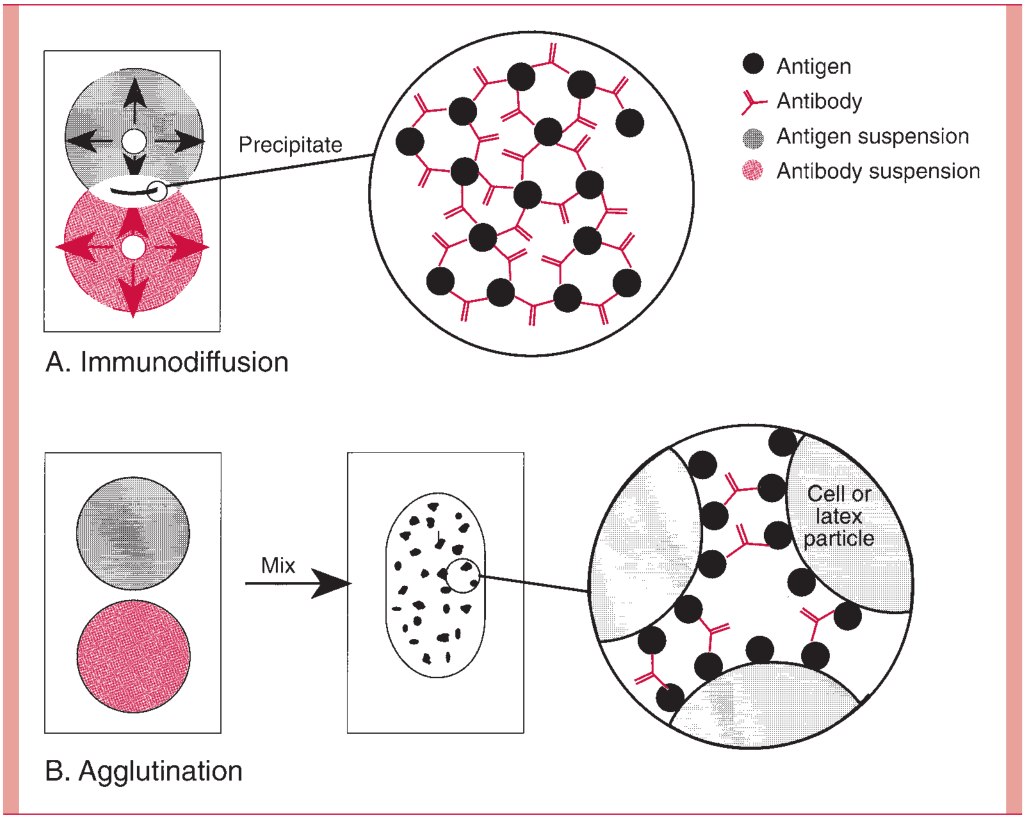



FIGURE 15–9
Immunodiffusion and agglutination. **A.** In immunodiffusion the antigen and antibody diffuse through a support matrix (eg, agarose). Where they reach optimal proportions a precipitin line is formed by the antigen–antibody complex. **B.** In agglutination the antigen–antibody reaction can be seen because one is on the surface of a relatively large particle. In the figure, the antigen is bound to the particle, but the reaction could be reversed.

Antigen–antibody precipitates demonstrated by immunodiffusion and CIE

In the immunodiffusion procedure, wells are cut in the agar and filled with antigen and antibody. One or more precipitin lines may be formed between the antigen and antibody wells; depending on the number of different antigen–antibody reactions occurring. **Counterimmunoelectrophoresis** (CIE) is immunodiffusion carried out in an electrophoretic field. The net effect is that antigen and antibody are rapidly brought together in the space between the wells to form a precipitin line.

## *Agglutination*

RBCs and latex particles coated with antigen or antibody enhance demonstration

Simple mixing on slide causes agglutination

The amount of antigen or antibody necessary to produce a visible immunologic reaction can be reduced if either is on the surface of a relatively large particle. This condition can be produced by fixing soluble antigens or antibody onto the surface of RBCs or microscopic latex particles (Fig 15–9B). Whole bacteria are large enough to serve as the particle if the antigen is present on the microbial surface. The relative proportions of antigen and antibody thus become less critical, and antigen–antibody reactions are detectable by agglutination when immune serum and particulate antigen, or particle-associated antibody and soluble antigen, are mixed on a slide. The process is termed bacterial agglutination, passive hemagglutination, or latex agglutination depending on the nature of the sensitized particle.

## *Neutralization*

Bacterium, virus, or toxin are mixed with antibody prior to addition to test system

Neutralization as commonly used takes some observable function of the agent, such as cytopathic effect of viruses or the action of a bacterial toxin, and neutralizes it. This is usually done by first reacting the agent with antibody, and then placing the antigen–antibody mixture into the test system. The steps involved are illustrated in Figure 15–10. In viral

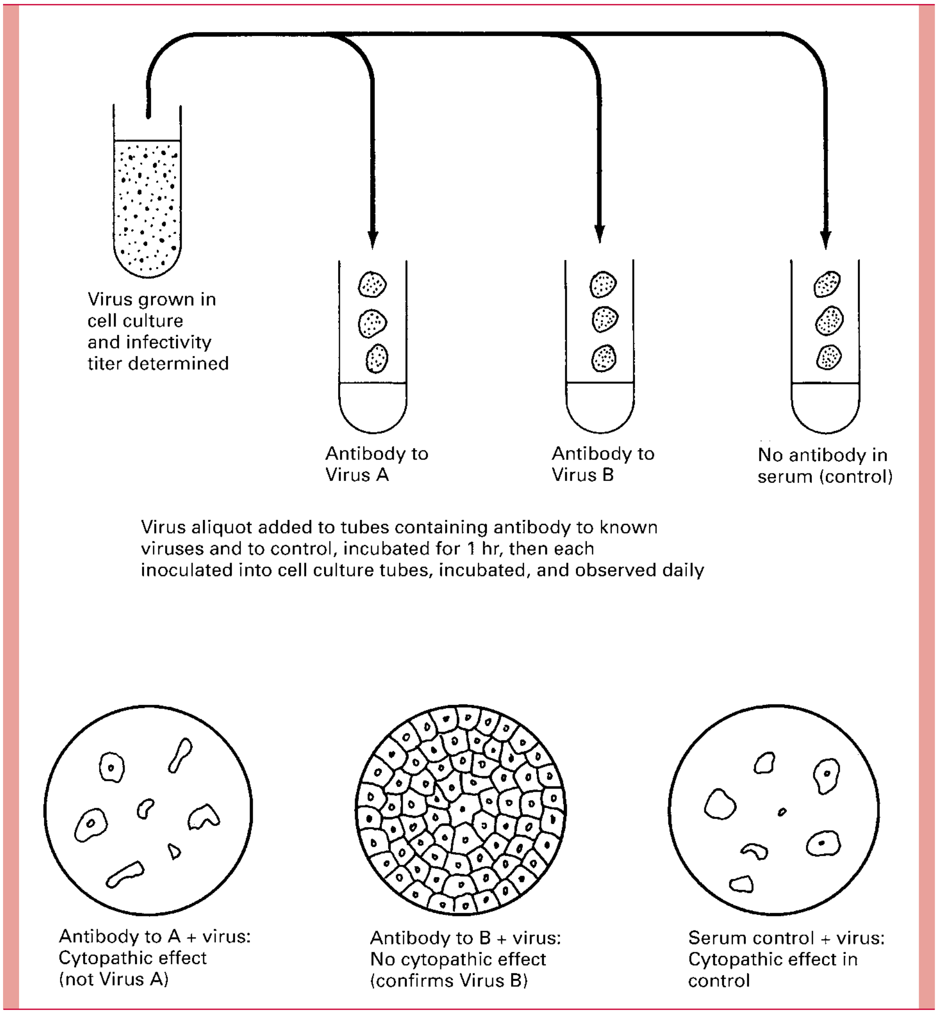



FIGURE 15–10
Identification of a virus isolate (cytopathic virus) as "Virus B."

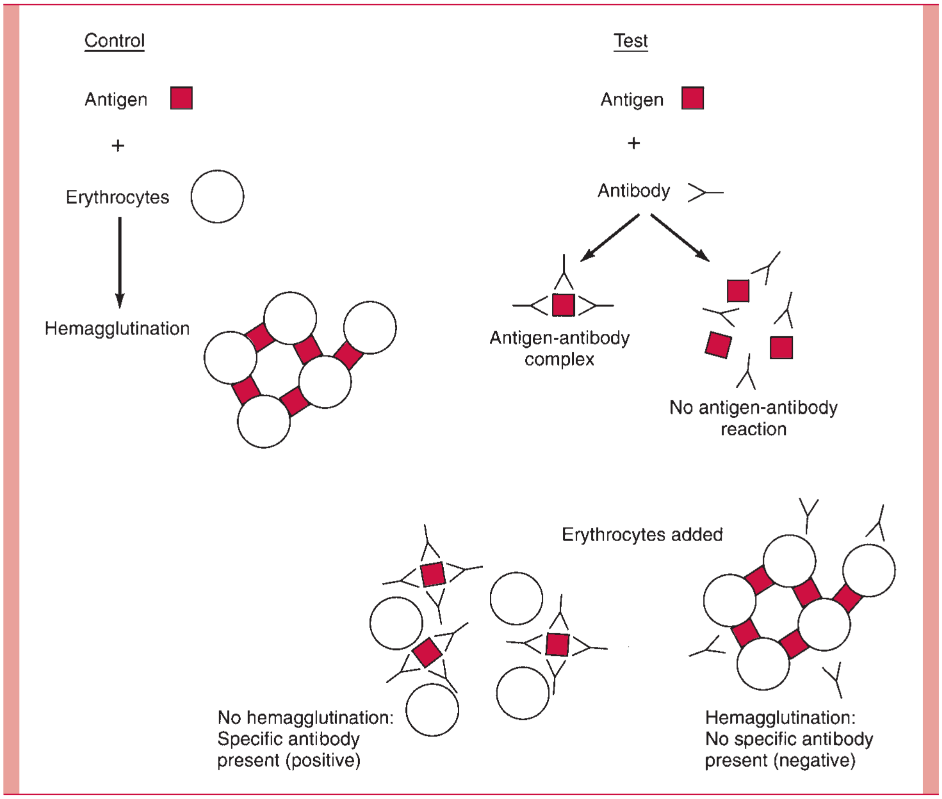



FIGURE 15–11
Hemagglutination inhibition for antibody detection (used when antigen agglutinates erythrocytes).

neutralization, a single antibody molecule can bind to surface components of the extracellular virus and interfere with one of the initial events of the viral multiplication cycle (adsorption, penetration, or uncoating). Some bacterial and viral agents directly bind to RBCs (hemagglutination). Neutralization of this reaction by antibody blocking of the receptor is called hemagglutination inhibition (Fig 15–11).

## *Complement Fixation*

Complement fixation assays depend on two properties of complement. The first is fixation (inactivation) of complement on formation of antigen–antibody complexes. The second is the ability of bound complement to cause hemolysis of sheep (RBCs coated with anti-sheep RBC antibody (sensitized RBCs). Complement fixation assays are performed in two stages: The test system reacts the antigen and antibody in the presence of complement; the indicator system, which contains the sensitized RBCs, detects residual complement. Hemolysis indicates that complement was present in the indicator system and therefore that antigen–antibody complexes were not formed in the test system. Primarily used to detect and quantitate antibody, complement fixation is gradually being replaced by simpler methods.

Action of complement on RBCs is used as indicator system

## *Labeling Methods*

Detection of antigen–antibody binding may be enhanced by attaching a label to one (usually the antibody) and detecting the label after removal of unbound reagents. The label may be a fluorescent dye (immunofluorescence), a radioisotope **(radioimmunoassay,** or **RIA),** or an enzyme **(enzyme immunoassay,** or **EIA)**. The presence or quantitation of antigen–antibody binding is measured by fluorescence, radioactivity, or the chemical reaction catalyzed by the enzyme.

Labeling antibody allows detection of fluorescence, radioactivity, or enzyme

***Immunofluorescence*** The most common labeling method in diagnostic microbiology is immunofluorescence (Fig 15–12), in which antibody labeled with a fluorescent dye, usually **fluorescein isothiocyanate (FITC)**, is applied to a slide of material that may contain

Light halo enhances microscopic visualization

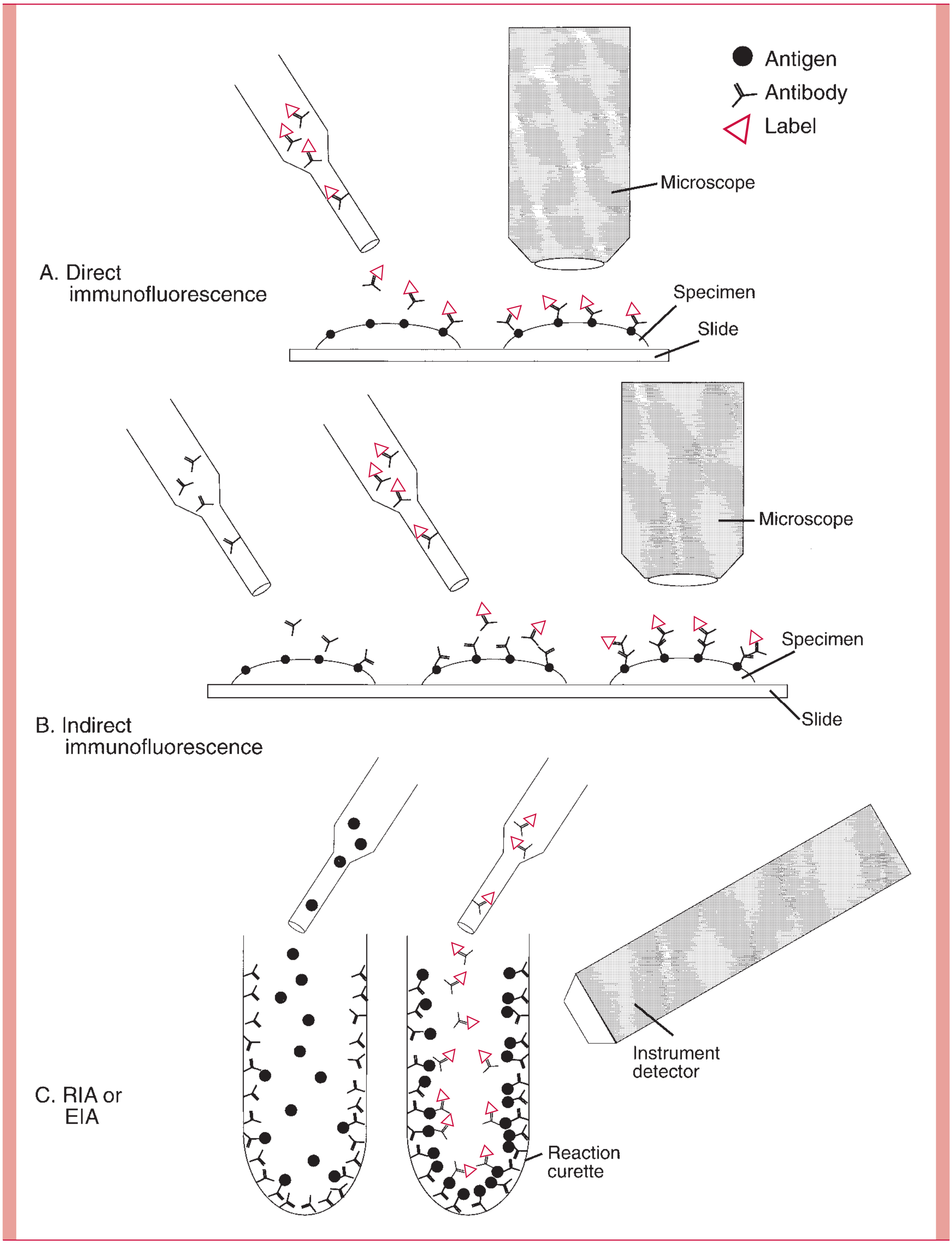



FIGURE 15–12

Labeling methods. **A.** In direct immunofluorescence the fluorescent compound is bound to the specific antibody and can be visualized as shown in Figure 15–2C. **B.** Indirect immunofluorescence has an extra step because the specific antibody is unlabeled. Its binding is detected by a second labeled antiglobulin antibody. **C.** A liquid phase immunoassay is shown. The antigen is "sandwiched" between an antibody bound to the tube and the labeled antibody. If the label is radioactive this is called radioimmunoassay (RIA), and if it is an enzyme it is called enzyme immunoassay (EIA). Many variations are possible.

the antigen sought. Under fluorescence microscopy, binding of the labeled antibody can be detected as a bright green halo surrounding bacterium, or in the case of viruses, as a fluorescent clump or on an infected cell. The method is called "direct" if the FITC is conjugated directly to the antibody with the desired specificity. In "indirect" immunofluorescence the specific antibody is not labeled, but its binding to an antigen is detected in an additional step using an FITC-labeled anti-immunoglobulin antibody that will bind to the specific antibody. Choice between the two approaches involves purely technical considerations.

Indirect methods use a second antibody

***Radioimmunoassay (RIA) and Enzyme Immunoassay (EIA)*** The labels used in RIA and EIA are more suitable for liquid phase assays and are particularly used in virology. They are also used in direct and indirect methods and many other ingenious variations such as the "sandwich" methods, so called because the antigen of interest is "trapped" between two antibodies (Fig 15–12C). These extremely sensitive techniques will be discussed further with regard to antibody detection.

Liquid phase RIA and EIA methods have many variants

## Serologic Classification

For most important antigens of diagnostic significance, antisera are commercially available. The most common test methods for bacteria are agglutination and immunofluorescence; and for viruses, neutralization. In most cases these methods subclassify organisms below the species level and thus are primarily of value for epidemiologic and research purposes. The terms "serotype" or "serogroup" are used together with numbers, letters, or Roman numerals with no apparent logic other than historical precedent. For a few genera the most fundamental taxonomic differentiation is serologic. This is the case with the streptococci (see Chapter 17), where an existing classification based on biochemical and cultural characteristics was superseded because a serologic classification scheme developed by Rebecca Lancefield correlated better with disease.

Antigenic systems classify below the species level

Serologic classification is primarily of epidemiologic value

Before these techniques can be applied to the diagnosis of specific infectious diseases, considerable study of the causative agent(s) is required. Antigen–antibody systems may vary in complexity from a single epitope to scores of epitopes on several macromolecular antigens whose chemical nature may or may not be known. The cause of the original 1976 outbreak of Legionnaires' disease (caused by *Legionella pneumophila*; see Chapter 26) was proven through the development of immune reagents that detected the bacteria in tissue and antibodies directed against the bacteria in the serum of patients. Now, more than 25 years later, there are more than a dozen serotypes and many additional species, each requiring specific immunologic reagents for antigen or antibody detection for diagnosis.

Proof of etiologic relationship depends on antigen detection

## Antibody Detection (Serology)

During infection—whether viral, bacterial, fungal, or parasitic—the host usually responds with the formation of antibodies, which can be detected by modification of any of the methods used for antigen detection. The formation of antibodies and their time course depends on the antigenic stimulation provided by the infection. The precise patterns vary depending on the antigens used, classes of antibody detected, and method. An example of temporal patterns of development and increase and decline in specific antiviral antibodies measured by different tests is illustrated in Figure 15–13. These responses can be used to detect evidence of recent or past infection. The test methods do not inherently indicate immunoglobulin class but can be modified to do so, usually by pretreatment of the serum to remove IgG to differentiate the IgM and IgG responses. Several basic principles must be emphasized:

Antibodies are formed in response to infection

Antibodies may indicate current, recent, or past infection

1. In an acute infection, the antibodies usually appear early in the illness, and then rise sharply over the next 10 to 21 days. Thus, a serum sample collected shortly after the onset of illness (acute serum) and another collected 2 to 3 weeks later (convalescent serum) can be compared quantitatively for changes in specific antibody content.

Paired specimens are compared

2. Antibodies can be quantitated by several means. The most common method is to dilute the serum serially in appropriate media and determine the maximal dilution that will still yield detectable antibody in the test system (eg, serum dilutions of 1:4, 1:8, and 1:16). The highest dilution that retains specific activity is called the antibody titer.

Titer is the highest serum dilution demonstrating activity

3. The interpretation of significant antibody responses (evidence of specific, recent infection) is most reliable when definite evidence of seroconversion is demonstrated; that is, detectable specific antibody is absent from the acute serum (or preillness serum, if available) but present in the convalescent serum. Alternatively, a fourfold or greater increase in antibody titer supports a diagnosis of recent infection; for example, an acute serum titer of 1:4 or less and a convalescent serum titer of 1:16 or greater would be considered significant.

Seroconversion or fourfold rise in titer most conclusive

4. In instances in which the average antibody titers of a population to a specific agent are known, a single convalescent antibody titer significantly greater than the expected mean may be used as supportive or presumptive evidence of recent infection. However, this finding is considerably less valuable than those obtained by comparing responses of acute and convalescent serum samples. An alternative and somewhat more complex method of serodiagnosis is to determine which major immunoglobulin subclass constitutes the major proportion of the specific antibodies. In primary infections, the IgM-specific response is often dominant during the first days or weeks after onset but is replaced progressively by IgG-specific antibodies; thus, by 1 to 6 months after infection, the predominant antibodies belong to the IgG subclass. Consequently, serum containing a high titer of antibodies of the IgM subclass would suggest a recent, primary infection.

Single titers may be useful in some circumstances

IgM responses indicate acute infection

The immunologic methods used to identify bacterial or viral antigens are applied to serologic diagnosis by simply reversing the detection system: that is, using a known rather than an unknown antigen to detect the presence of an antibody. The methods of serologic diagnosis to be used are selected on the basis of their convenience and applicability to the antigen in question. As shown in Figure 15–13, the temporal relationships of antibody response to infection vary according to the method used. Of the methods for measuring antigen–antibody interaction discussed previously, those now used most frequently for serologic diagnosis are agglutination, RIA, and EIA (see Figs 15–9 and 15–11).

Experience with systems and temporal relationships aids interpretation

## *Western Blot*

The Western blot immunoassay is another technique that is now commonly employed to detect and confirm the specificity of antibodies to a variety of epitopes. Its greatest use has been in the diagnosis of HIV infections (see Chapter 42), in which virions are electrophoresed in a polyacrylamide gel to separate the protein and glycoprotein components

Western blot confirms specificity of antibodies for protein components of the agent (eg, HIV)

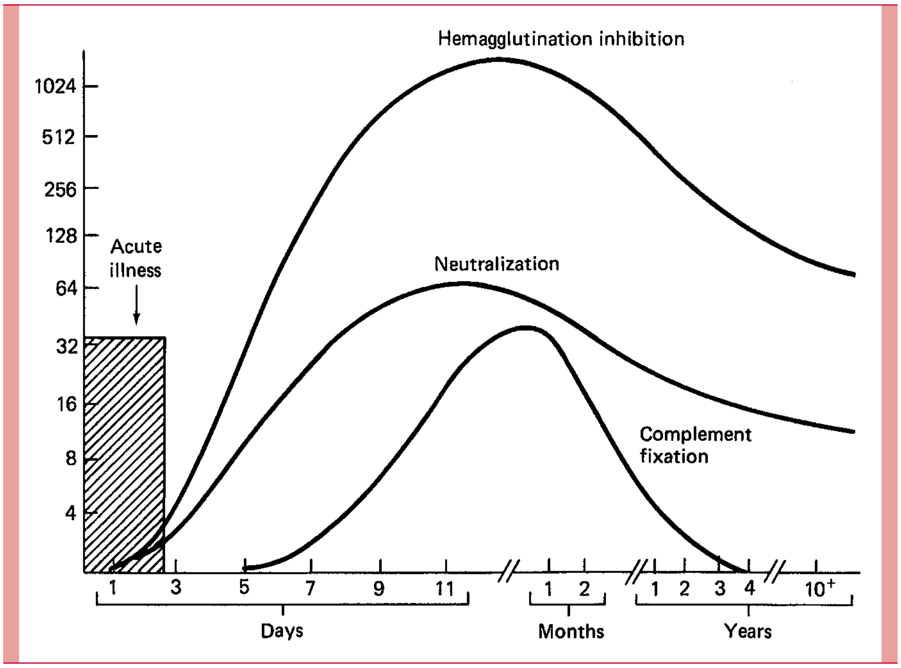



FIGURE 15–13
Examples of patterns of antibody responses to an acute infection, measured by three different methods.

and then transferred onto nitrocellulose. This is then incubated with patient serum, and antibody to the different viral components is detected by using an antihuman globulin IgG antibody conjugated with an enzyme label.

### Antigen Detection

Theoretically, any of the methods described for detecting antigen–antibody interactions can be applied directly to clinical specimens. The most common of these is immunofluorescence, in which antigen is detected on the surface of the organism or in cells present in the infected secretion. The greatest success with this approach has been in respiratory infections where a nasopharyngeal, throat washing, sputum, or bronchoalveolar lavage specimen may contain bacteria or viral aggregates in sufficient amount to be seen microscopically. Although the fluorescent tag makes it easier to find organisms, these methods are generally not as sensitive as culture. With some genera and species, the immunofluorescent detection of antigens in clinical material provides the most rapid means of diagnosis, as with *Legionella* and respiratory syncytial virus.

Immunofluorescence detects agents in respiratory secretions

Another approach is to detect free antigen released by the organism into body fluids. This offers the possibility of bypassing direct examination, culture, and identification tests to achieve a diagnosis. Success requires a highly specific antibody, a sensitive detection method, and the presence of the homologous antigen in an accessible body fluid. The latter is an important limitation, because not all organisms release free antigen in the course of infection. At present, diagnosis by antigen detection is limited to some bacteria and fungi with polysaccharide capsules (eg, *Haemophilus influenzae*), *Chlamydia*, and to certain viruses. The techniques of agglutination with antibody bound to latex particles, CIE, RIA, and EIA are used to detect free antigen in serum, urine, cerebrospinal fluid, and joint fluid. Live organisms are not required for antigen detection, and these tests may still be positive when the causative organism has been eliminated by antimicrobial therapy. The procedures can yield results within an hour or two, sometimes within a few minutes. This feature is attractive for office practice, because it allows diagnostic decisions to be made during the patient's visit. A number of commercial products detect group A streptococci in sore throats with over 90% sensitivity; however, because these tests are less sensitive than culture, negative results must be confirmed by culture.

Soluble antigens may be detected in body fluids

Rapid detection can replace culture

## NUCLEIC ACID ANALYSIS

Analysis of the DNA or RNA of microorganisms is the basis of newer taxonomic studies and increasingly applied to diagnostic and epidemiologic work. It is also possible to use cloned or synthesized nucleic acid probes to detect genes or smaller nucleotide sequences specific for a variety of bacterial, viral, and other infectious agents. As with antigen–antibody reactions, a variety of methods have been developed for analysis of nucleic acids. Those relevant to the study of infectious diseases are briefly summarized below. The student is referred to textbooks of molecular biology for more complete coverage. DNA is a hardy molecule that will withstand fairly harsh chemical treatment. RNA is more fragile, primarily because it is readily digested by the RNAse enzymes commonly found in biologic systems. The extraction process for bacteria and fungi involves breaking open the cells, precipitating the protein, and extracting the nucleic acid with ethanol. Viral procedures are similar except that much of the separation and concentration may be accomplished by ultracentrifugation.

DNA is extracted from bacteria and fungi

Viral DNA and RNA are concentrated by ultracentrifugation

### Methods of Nucleic Acid Analysis

#### *Agarose Gel Electrophoresis*

Nucleic acids may be separated in an electrophoretic field in an **agarose** (highly purified agar) gel. The speed of migration depends on size, with the smaller molecules moving faster and appearing at the bottom (end) of the gel. This method is able to separate DNA fragments in the range of 0.1 to 50 kilobases, which is far below the size of bacterial genomes but includes some naturally occurring genetic elements such as bacterial plasmids (Fig 15–14). A variant of agarose gel electrophoresis, **pulsed field electrophoresis**, alternates the orientation of electrical field in a fashion that allows resolution of much larger DNA fragments.

Agarose gel electrophoresis separates DNA fragments or plasmids based on size

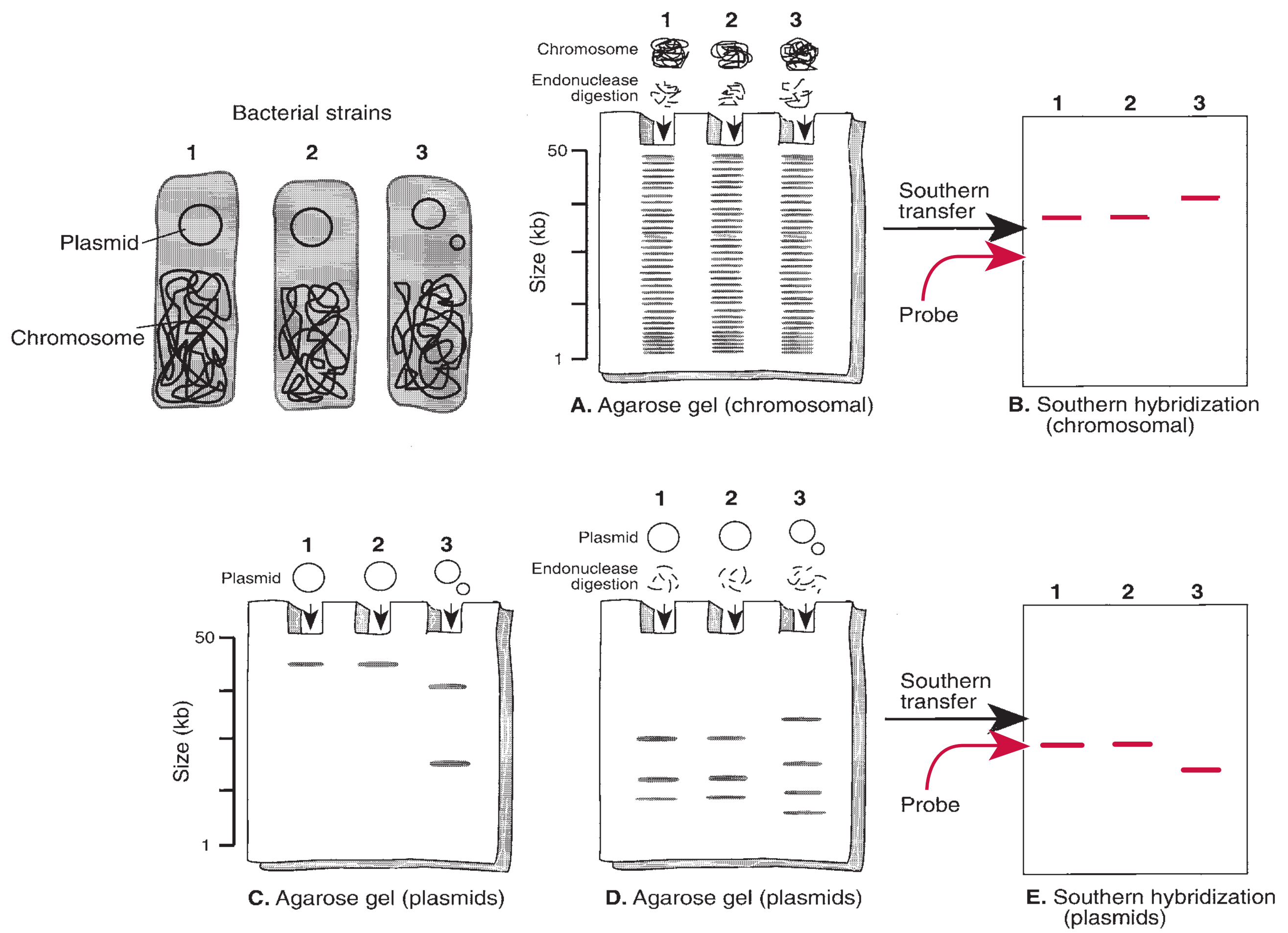



FIGURE 15–14
Molecular diagnostic methods. Three bacterial strains of the same species are shown each with chromosome and plasmid(s). **A.** The chromosomal DNA of each strain is isolated, digested with a restriction endonuclease, and separated by agarose gel electrophoresis. An almost continuous range of fragment sizes is generated for each strain, making them difficult to distinguish. **B.** The restriction fragments in A are transferred to a membrane (Southern transfer) and hybridized with a probe. The probe binds to a single fragment from each strain, but the larger size of the fragment from strain 3 indicates variation in restriction sites and thus a genomic difference between it and strains 1 and 2. **C.** Plasmids from each strain are isolated and separated in the same manner as A. The results show a plasmid of the same size from 1 and 2. Strain 3 has two plasmids each of a different size than strains 1 and 2. **D.** The same plasmids are restriction digested prior to electrophoresis. The plasmids from strains 1 and 2 show three fragments of identical size, proving they are identical. The plasmids of strain 3 appear unrelated. **E.** The fragments in D are transferred and reacted with a probe. The positive result with the largest of the strain 1 and 2 fragments confirms their relatedness. The positive hybridization with one of the strain 3 fragments suggests that it contains at least some DNA that is homologous to the plasmid from strains 1 and 2.

## *Restriction Endonuclease Digestion*

Restriction endonuclease digestion refines electrophoretic analysis of DNA

Restriction endonucleases are enzymes that recognize specific nucleotide sequences in DNA molecules and digest (cut) them at all sites at which the sequence appears. A large number of these enzymes have been isolated from bacterial strains and are commercially available together with information on the sequences they recognize. While the four- to eight-base pair sequences recognized by these endonucleases are not unique to any one organism, their spacing along the chromosome or other genomic structure may be. The

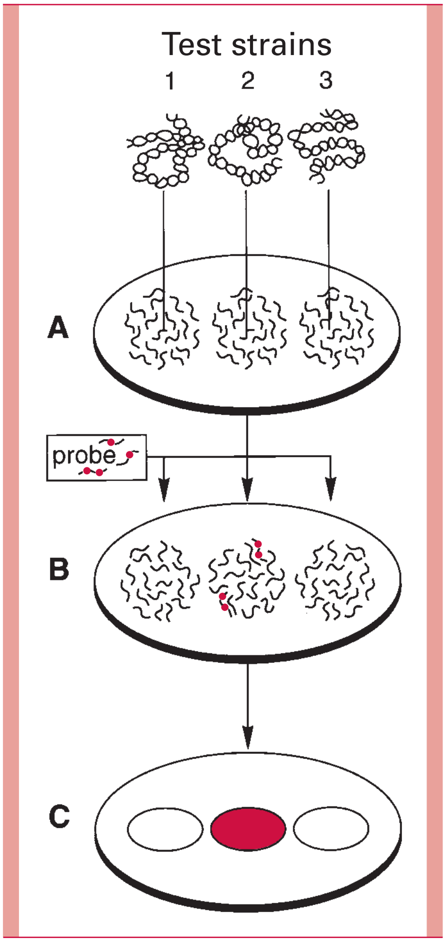



FIGURE 15–15
DNA probe detection. **A.** Chromosomal and/or plasmid DNA from three unknown strains is fragmented, denatured, and bound to filters. **B.** The probe ( ) is a small DNA fragment labeled with a radioactive or other marker. It is allowed to react with the single-stranded test DNAs on the filter and binds wherever homologous sequences are found. **C.** Probe that has hybridized with test DNA is detected on the filter by an appropriate test for the marker. Test strain 2 contains sequences homologous to the probe and thus gives a positive reaction.

size of fragments generated by endonuclease digestion of DNA molecules may be compared by agarose gel electrophoresis (Fig 15–15).

## *DNA Hybridization*

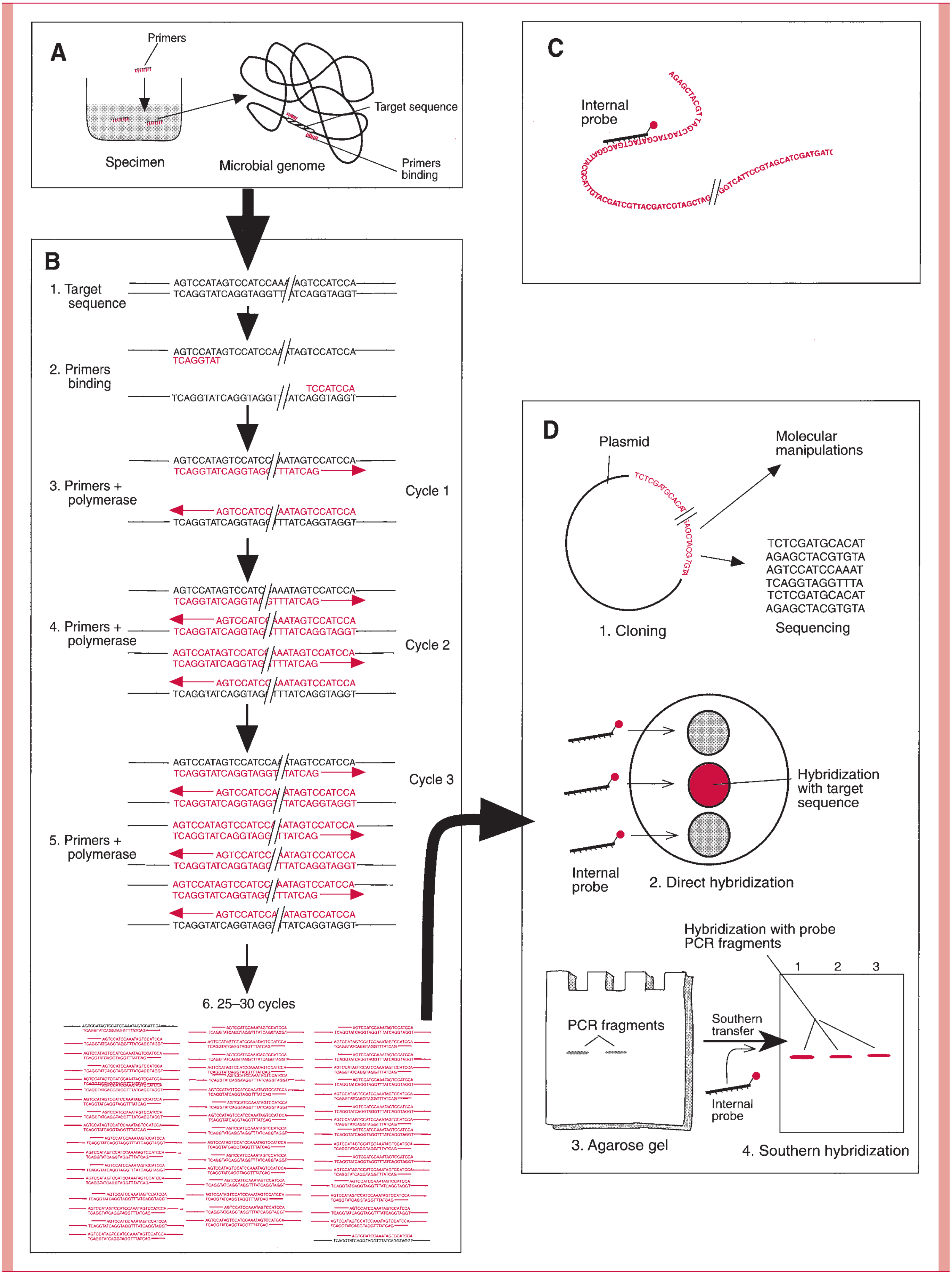
If the DNA double helix is opened, leaving single-stranded (denatured) DNA, the nucleotide bases are exposed and thus available to interact with other single-stranded nucleic acid molecules. If complementary sequences of a second DNA molecule are brought into physical contact with the first, they will hybridize to it, forming a new double-stranded molecule in that area. A variety of methods are in use that allow hybridization to take place between two or more nucleic acid molecules. The reaction mixtures vary from tiny probes to the entire genome of an organism. Most immobilize the single-stranded target DNA on a membrane to prevent it from rehybridizing with its own complementary strand, but liquid phase assays have also been developed. A variant in which the DNA is separated by agarose gel electrophoresis before binding to the membrane is called **Southern hybridization.**

DNA hybridization methods allow DNA from different sources to combine

## *Polymerase Chain Reaction*

The polymerase chain reaction (PCR) is an amplification technique that allows the detection and selective replication of a targeted portion of the genome. The technique uses special DNA polymerases that through alternate changes in test conditions such as temperature can be manipulated to initiate replication in either the 3′ or 5′ direction. The specificity is provided by primers that recognize a pair of unique sites on the chromosome so that the DNA between them can be replicated by repetitive cycling of the test conditions. Because each newly synthesized fragment can serve as the template for its own replication, the amount of DNA doubles exponentially with each cycle (Fig 15–16).

PCR amplifies targeted segments of the genome


A
Primers
Specimen
Microbial genome
Target sequence
Primers
binding
B
1. Target
sequence
AGTCCATAGTCCATCCAAA/ /AGTCCATCCA
TCAGGTATCAGGTAGGTT/ /ATCAGGTAGGT
2. Primers
binding
AGTCCATAGTCCATCCA/ /TAGTCCATCCA
TCAGGTAT
TCCATCCA
TCAGGTATCAGGTAGGT/ /ATCAGGTAGGT
3. Primers +
polymerase
AGTCCATAGTCCATCC/ /AATAGTCCATCCA
TCAGGTATCAGGTAGG/ /TTATCAG
AGTCCATCC/ /AATAGTCCATCCA
TCAGGTATCAGGTAGG/ /TTATCAGGTAGGT
Cycle 1
4. Primers +
polymerase
AGTCCATAGTCCATC/ /AAATAGTCCATCCA
TCAGGTATCAGGTAG/ /GTTTATCAG
AGTCCATCC/ /AAATAGTCCATCCA
TCAGGTATCAGGTAG/ /TTTATCAGGTAGGT
AGTCCATAGTCCATCC/ /AAATAGTCCATCCA
TCAGGTATCAGGTAG/ /TTTATCAG
AGTCCATCC/ /AATAGTCCATCCA
TCAGGTATCAGGTAG/ /TTTATCAGGTAGGT
Cycle 2
5. Primers +
polymerase
AGTCCATAGTCCATCCA/ /TAGTCCATCCA
TCAGGTATCAGGTAGGT/ /TATCAG
AGTCCATCCA/ /ATAGTCCATCCA
TCAGGTATCAGGTAGGT/ /TATCAGGTAGGT
AGTCCATAGTCCATCC/ /ATAGTCCATCCA
TCAGGTATCAGGTAGG/ /TTATCAG
AGTCCATCC/ /AATAGTCCATCCA
TCAGGTATCAGGTAGG/ /TTATCAGGTAGGT
AGTCCATAGTCCATCC/ /AATAGTCCATCCA
TCAGGTATCAGGTAGG/ /TTATCAG
AGTCCATCCA/ /ATAGTCCATCCA
TCAGGTATCAGGTAGG/ /TATCAGGTAGGT
Cycle 3
6. 25–30 cycles
C
Internal
probe
D
Plasmid
Molecular
manipulations
TCTCGATGCACAT
AGAGCTACGTGTA
AGTCCATCCAAAT
TCAGGTAGGTTTA
TCTCGATGCACAT
AGAGCTACGTGTA
1. Cloning
Sequencing
Hybridization
with target
sequence
Internal
probe
2. Direct hybridization
Hybridization with probe
PCR fragments
1
2
3
PCR fragments
Southern
transfer
Internal
probe
3. Agarose gel
4. Southern hybridization

### *Nucleic Acid Sequence Analysis*

For some time, it has been possible to chemically determine the exact nucleotide sequence of genomic segments or cloned genes. Published sequences are systematically entered into computer databases such as GenBank and are widely available for analysis by computer software designed to solve a wide variety of problems. Conversely, given the known sequence, short segments of DNA can be synthesized for use as probes or primers. It is even possible to compare a sequenced gene or putative probe against all known sequences using the computer, an "experiment" that would be impossible in the laboratory.

Nucleic acid sequence data is available in computerized formats

## Application of Nucleic Acid Methods to Infectious Diseases

### *Bacterial and Viral Genomic Sizing*

The only intact genetic elements of infectious agents that are small enough to be directly detected and sized by agarose gel electrophoresis are bacterial plasmids. Not all bacterial species typically harbor plasmids, but those that do may carry one or a number of plasmids ranging in size from less than 1 to over 50 kilobases. This diversity makes the presence or absence, number, and sizes of plasmids of considerable value in differentiating strains for epidemiologic purposes. Because plasmids are not stable components of the bacterial genome, plasmid analysis also has the element of a timely "snapshot" of the circumstances of a disease outbreak. The specificity of these results can be improved by digesting the plasmids with restriction endonucleases prior to electrophoresis. Two plasmids of the same size from different strains may not be the same, but if an identical pattern of fragments is generated from the digestion, they almost certainly are. These principles are illustrated Figure 15–14 and their application to an outbreak is shown in Figures 4–12 and 4–13.

Number and size of plasmids differentiates strains

Endonuclease digestion of plasmids refines their comparison

Because of their larger size, the chromosomes of bacteria must be digested with endonucleases to resolve them on gels. For viruses the outcome is much like that with plasmids, depending on the genomic size and the endonuclease used. Digested bacterial chromosomes can be compared in this manner, but the number of fragments is very large and the patterns complex. The combined use of endonucleases, which make infrequent cuts, and pulsed-field electrophoresis can produce a comparison comparable to that possible with plasmids. This approach is also used for analysis of the multiple chromosomes of fungi and parasites.

Bacterial chromosomes must be digested prior to electrophoresis

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FIGURE 15–16
Diagnostic applications of the polymerase chain reaction (PCR). **A.** A clinical specimen (eg, pus, tissue) contains DNA from many sources as well as the chromosome of the organism of interest. If the DNA strands are separated (denatured), the PCR primers can bind to their target sequences in the specimen itself. **B.** Amplification of the target sequence by PCR. (1) The target sequence is shown in its native state. (2) The DNA is denatured, allowing the primers to bind where they find the homologous sequence. (3) In the presence of the special DNA polymerase, new DNA is synthesized from both strands in the region between the primers. (4 to 6) Additional cycles are added by temperature control of the polymerase with each new sequence acting as the template for another. The DNA doubles with each cycle. After 25 to 30 cycles enough DNA is present to analyze diagnostically. **C.** Internal probe. The amplified target sequence is shown. A probe can be designed to bind to a sequence located between (internal to) the primers. **D.** Analysis of PCR amplified DNA. (1) The amplified sequence can be cloned into a plasmid vector. In this form, a variety of molecular manipulations or sequencing may be carried out. (2) Direct hybridizations usually make use of an internal probe. The example shows three specimens, each of which went through steps **A** and **B**. Following amplification each was bound to a separate spot on a filter (dot blot). The filter is then reacted with the internal probe to detect the PCR-amplified DNA. The result shows that only the middle specimen contained the target sequence. (3) The amplified DNA may be detected directly by agarose gel electrophoresis. The example shows detection of amplified fragments in two of three lanes on the gel. (4) The sensitivity of detection may be increased by use of the internal probe following Southern transfer. The example shows detection of a third fragment of the same size that was not seen on the original gel because the amount of DNA was too small.

## *DNA Probes*

Probes may be cloned or synthesized from known sequences

A "probe" is a fragment of DNA that has been cloned or otherwise recovered from a genomic or plasmid source. It may contain a gene of known function or simply sequences empirically found to be useful for the application in question. In some cases, the probe is synthesized as a single chain of nucleotides (oligonucleotide probe) from known sequence data. The probes are labeled with a radioisotope or other marker and used in hybridization reactions either to detect the homologous sequences in unknown specimens (see Fig 15–15) or to further refine gel electrophoresis findings (see Fig 15–14). In the latter instance, Southern hybridizations are used to retain knowledge of the size of fragments involved. For example, the information that the same gene is present in each of two strains but in different size restriction fragments is evidence for a genomic difference between the two strains (Fig 15–14B).

Probes can detect DNA of pathogen directly in clinical specimens

The diagnostic use of DNA probes is to detect or identify microorganisms by hybridization of the probe to homologous sequences in DNA extracted from the entire organism. A number of probes have been developed that will quickly and reliably identify organisms already isolated in culture. The application of probes for detection of infectious agents directly in clinical specimens such as blood, urine, and sputum is more difficult, because most of the systems developed to date are not as sensitive as culture and are more expensive. However, this approach offers the potential for rapid diagnosis and the detection of characteristics not possible by routine methods. For example, a bacterial toxin gene probe can demonstrate both the presence of the related organism and its toxigenicity without the need for culture.

## *Applications of Polymerase Chain Reaction (PCR)*

PCR combined with probes gives the greatest sensitivity

The amplification power of the PCR offers a solution for the sensitivity problems inherent in the direct application of probes (Fig 15–16). Although the nucleic acid segment amplified by PCR can be seen directly on a gel, the greatest sensitivity and specificity are achieved when probe hybridization is carried out following PCR. This approach has been successful for a wide range of infectious agents and awaits only further resolution of practical problems for wider use.

PCR from tissue allows study of organisms that cannot be cultured

Another creative use of PCR has been in the study of infectious agents seen in tissue but not grown in culture. PCR primers derived from sequences known to be highly conserved among bacteria, such as ribosomal RNA, have been applied to tissue specimens. The amplification produces enough DNA to clone and sequence. This sequence can then be compared with sequences published for other organisms using computers. Thus, taxonomic relationships can be inferred for an organism that has never been isolated.

## *Ribotyping*

Ribotyping refines comparison of chromosomal endonuclease digestion patterns

Ribotyping also makes use of the conserved nature of bacterial ribosomal RNA and of the ability of RNA to hybridize to DNA under certain conditions. Labeled ribosomal RNA of one organism can be hybridized with restriction endonuclease–digested chromosomal DNA of another. In this case, ribosomal RNA is being used as a massive probe of restriction fragments separated by electrophoresis. Hybridization to multiple fragments is common, but if the organisms are genetically different, the restriction fragments, which contain the ribosomal RNA sequences, will vary in size. The pattern of bands produced by epidemiologically related strains can then be compared side by side.

## *Genomic Analysis*

Percent DNA homology is now a primary tool for taxonomic comparisons

DNA homology techniques hybridize the total genomic DNA of one organism to that of another in a manner demonstrated in Figure 15–17. The relatedness of strains can be expressed as a percent homology. Strains related at the species level should show homology in the 60 to 90% range, whereas strains with increasing taxonomic divergence show progressively less homology. These findings are now a major factor in decisions on the

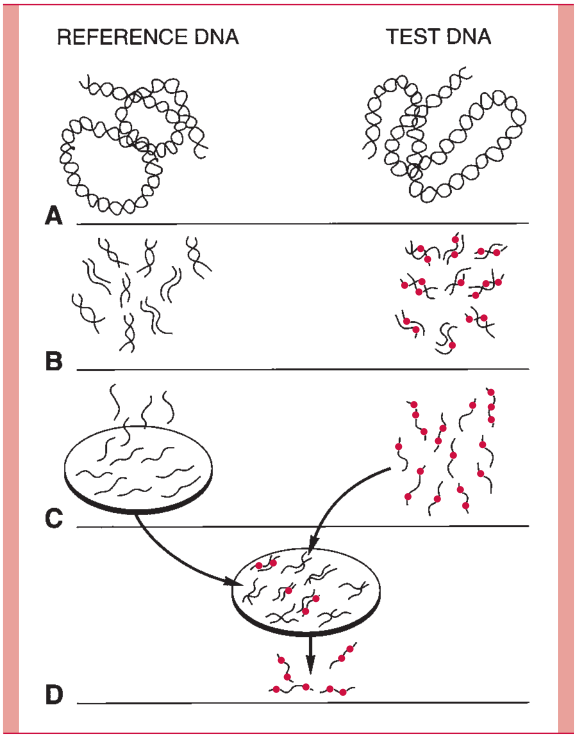


FIGURE 15–17
DNA–DNA homology. **A.** Double-stranded chromosomal DNA from a test strain is to be compared with a reference strain of the same or another species. **B.** Both DNAs are fragmented and denatured. The test DNA is labeled ( ) with a radioisotope or some other marker. **C.** The denatured (single-stranded) reference DNA is bound to a support matrix such as a nitrocellulose or nylon filter, thus leaving the nucleotide bases available for pairing. **D.** The labeled test DNA is reacted with the material on the filter allowing homologous sequences to pair (hybridize) with the reference DNA. Nonhomologous DNA is washed away, and the amount of bound label measured. The percentage homology of the test to the reference DNA is determined from the ratio of bound to unbound label.

taxonomic classification of all microorganisms, allowing species, genus, and higher taxonomic groupings to be assessed by means that are not subject to the phenotypic variation inherent with classical methods. Sequence analysis of mutations in genes related to the action of drugs used in the treatment of AIDS can now be used to study and even predict emergence of drug resistance.

Drug resistance can be predicted by sequence analysis

## SUMMARY

The application of some combination of the principles described in this chapter is appropriate to the diagnosis of any infectious disease. The usefulness of any individual method differs among infectious agents due to biologic variation and uneven study. In general, for agents that can be grown in vitro, culture remains the "gold standard" as both the most sensitive and specific method. Molecular methods have the potential to replace culture once they are more fully evaluated and cost effective.

## ADDITIONAL READING

*Cumulative Techniques and Procedures in Clinical Microbiology (CUMITECH).* Washington, DC: American Society for Microbiology. CUMITECH is a series of 10- to 25-page pamphlets, each of which covers important topics related to diagnostic microbiology (eg, blood cultures, urinary tract infections, antimicrobial susceptibility testing). Each

pamphlet is jointly written by at least three authors representing the clinical as well as the laboratory viewpoint and includes clinical, specimen collection, isolation, and identification recommendations for all agents pertinent to the topic.

Murray PR (ed). *Manual of Clinical Microbiology*, 7th ed. Washington, DC: American Society for Microbiology; 1999. A widely used comprehensive text written for pathologists and medical technologists includes clinical bacteriology, mycology, parasitology, and virology.

Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. *N Engl J Med* 1992;327:293–301. A wonderful example of taxonomy using molecular methods alone.

## APPENDIX 15–1. SOME MEDIA USED FOR ISOLATION OF BACTERIAL PATHOGENS

| Medium | Uses |
|---|---|
| **General-purpose Media** | |
| Nutrient broths (eg, Soybean–Casein digest broth) | Most bacteria, particularly when used for blood culture |
| Thioglycolate broth | Anaerobes, facultative bacteria |
| Blood agar | Most bacteria (demonstrates hemolysis) |
| Chocolate agar | Most bacteria, including fastidious species (eg, *Haemophilus*) |
| **Selective Media** | |
| MacConkey agar | Nonfastidious Gram-negative rods |
| Hektoen-enteric agar | *Salmonella* and *Shigella* |
| Selenite F broth | *Salmonella* enrichment |
| **Special-purpose Media** | |
| Löwenstein–Jensen medium, Middlebrook agar | *Mycobacterium tuberculosis* and other mycobacteria (selective) |
| Martin–Lewis medium | *Neisseria gonorrhoeae* and *N. meningitidis* (selective) |
| Fletcher medium (semisolid) | Leptospira (nonselective) |
| Tinsdale agar | *Corynebacterium diphtheriae* (selective) |
| Charcoal agar | *Bordetella pertussis* (selective) |
| Buffered charcoal–yeast extract agar | *Legionella* species (nonselective) |
| *Campylobacter* blood agar | *Campylobacter jejuni* (selective) |
| Thiosulfate-citrate-bile-sucrose agar (TCBS) | *Vibrio cholerae* and *V. parahaemolyticus* (selective) |

## APPENDIX 15–2. CHARACTERISTICS OF COMMONLY USED BACTERIOLOGIC MEDIA

1. **Nutrient broths.** Some form of nutrient broth is used for culture of all direct tissue or fluid samples from sites that are normally sterile to obtain the maximum culture sensitivity. Selective or indicator agents are omitted to prevent inhibition of more fastidious organisms.
2. **Blood agar.** The addition of defibrinated blood to a nutrient agar base enhances the growth of some bacteria, such as streptococci. It often yields distinctive colonies and provides an indicator system for hemolysis. Two major types of hemolysis are seen:

$\beta$-hemolysis, a complete clearing of red cells from a zone surrounding the colony; and $\alpha$-hemolysis, which is incomplete (that is, intact red cells are still present in the hemolytic zone), but shows a green color caused by hemoglobin breakdown products. The net effect is a hazy green zone extending 1 to 2 mm beyond the colony. A third type, $\alpha'$-hemolysis, produces a hazy, incomplete hemolytic zone similar to that caused by $\alpha$-hemolysis, but without the green coloration.

3. **Chocolate agar.** If blood is added to molten nutrient agar at about 80°C and maintained at this temperature, the red cells are gently lysed, hemoglobin products are released, and the medium turns a chocolate brown color. The nutrients released permit the growth of some fastidious organisms, such as *Haemophilus influenzae,* that fail to grow on blood or nutrient agars. This quality is particularly pronounced when the medium is further enriched with vitamin supplements. Given the same incubation conditions, any organism that grows on blood agar will also grow on chocolate agar.
4. **Martin–Lewis medium.** A variant of chocolate agar, Martin–Lewis medium is a solid medium selective for the pathogenic *Neisseria* (*N. gonorrhoeae* and *N. meningitidis*). Growth of most other bacteria and fungi in the genital or respiratory flora is inhibited by the addition of antimicrobics. One formulation includes vancomycin, colistin, trimethoprim, and anisomycin.
5. **MacConkey agar.** MacConkey agar is both a selective and an indicator medium for Gram-negative rods, particularly members of the family Enterobacteriaceae and the genus *Pseudomonas.* In addition to a peptone base, the medium contains bile salts, crystal violet, lactose, and neutral red as a pH indicator. The bile salts and crystal violet inhibit Gram-positive bacteria and the more fastidious Gram-negative organisms, such as *Neisseria* and *Pasteurella*. Gram-negative rods that grow and ferment lactose produce a red (acid) colony, often with a distinctive colonial morphology.
6. **Hektoen enteric agar.** The Hektoen medium is one of many highly selective media developed for the isolation of *Salmonella* and *Shigella* species from stool specimens. It has both selective and indicator properties. The medium contains a mixture of bile, thiosulfate, and citrate salts that inhibits not only Gram-positive bacteria, but members of the Enterobacteriaceae other than *Salmonella* and *Shigella* that appear among the normal flora of the colon. The inhibition is not absolute; recovery of *Escherichia coli* is reduced 1000- to 10,000-fold relative to that on nonselective media, but there is little effect on growth of *Salmonella* and *Shigella.* Carbohydrates and a pH indicator are also included to help to differentiate colonies of *Salmonella* and *Shigella* from those of other enteric Gram-negative rods.
7. **Anaerobic media.** In addition to meeting atmospheric requirements, isolation of some strictly anaerobic bacteria on blood agar is enhanced by reducing agents such as L-cysteine and by vitamin enrichment. Sodium thioglycolate, another reducing agent, is often used in broth media. Plate media are made selective for anaerobes by the addition of aminoglycoside antibiotics, which are active against many aerobic and facultative organisms but not against anaerobic bacteria. The use of selective media is particularly important with anaerobes because they grow slowly and are commonly mixed with facultative bacteria in infections.
8. **Highly selective media.** Media specific to the isolation of almost every important pathogen have been developed. Many will allow only a single species to grow from specimens with a rich normal flora (eg, stool). The most common of these media are listed in Appendix 15–1; they are discussed in greater detail in following chapters.

## APPENDIX 15–3. COMMON BIOCHEMICAL TESTS FOR MICROBIAL IDENTIFICATION

1. **Carbohydrate breakdown.** The ability to produce acidic metabolic products, fermentatively or oxidatively, from a range of carbohydrates (eg, glucose, sucrose, and lactose) has been applied to the identification of most groups of bacteria. Such tests are crude and imperfect in defining mechanisms, but have proved useful for taxonomic purposes. More recently, gas chromatographic identification of specific

short-chain fatty acids produced by fermentation of glucose has proved useful in classifying many anaerobic bacteria.

2. **Catalase production.** The enzyme catalase catalyzes the conversion of hydrogen peroxide to water and oxygen. When a colony is placed in hydrogen peroxide, liberation of oxygen as gas bubbles can be seen. The test is particularly useful in differentiation of staphylococci (positive) from streptococci (negative), but also has taxonomic application to Gram-negative bacteria.
3. **Citrate utilization.** An agar medium that contains sodium citrate as the sole carbon source may be used to determine ability to use citrate. Bacteria that grow on this medium are termed **citrate positive.**
4. **Coagulase.** The enzyme coagulase acts with a plasma factor to convert fibrinogen to a fibrin clot. It is used to differentiate *Staphylococcus aureus* from other, less pathogenic staphylococci.
5. **Decarboxylases and deaminases.** The decarboxylation or deamination of the amino acids lysine, ornithine, and arginine is detected by the effect of the amino products on the pH of the reaction mixture or by the formation of colored products. These tests are used primarily with Gram-negative rods.
6. **Hydrogen sulfide.** The ability of some bacteria to produce $H_2S$ from amino acids or other sulfur-containing compounds is helpful in taxonomic classification. The black color of the sulfide salts formed with heavy metals such as iron is the usual means of detection.
7. **Indole.** The indole reaction tests the ability of the organism to produce indole, a benzopyrrole, from tryptophan. Indole is detected by the formation of a red dye after addition of a benzaldehyde reagent. A spot test can be done in seconds using isolated colonies.
8. **Nitrate reduction.** Bacteria may reduce nitrates by several mechanisms. This ability is demonstrated by detection of the nitrites and/or nitrogen gas formed in the process.
9. **O-Nitrophenyl-$\beta$-D-galactoside (ONPG) breakdown.** The ONPG test is related to lactose fermentation. Organisms that possess the $\beta$-galactoside necessary for lactose fermentation but lack a permease necessary for lactose to enter the cell are ONPG positive and lactose negative.
10. **Oxidase production.** The oxidase tests detect the *c* component of the cytochrome–oxidase complex. The reagents used change from clear to colored when converted from the reduced to the oxidized state. The oxidase reaction is commonly demonstrated in a spot test, which can be done quickly from isolated colonies.
11. **Proteinase production.** Proteolytic activity is detected by growing the organism in the presence of substrates such as gelatin or coagulated egg.
12. **Urease production.** Urease hydrolyzes urea to yield two molecules of ammonia and one of $CO_2$. This reaction can be detected by the increase in medium pH caused by ammonia production. Urease-positive species vary in the amount of enzyme produced; bacteria can thus be designated as positive, weakly positive, or negative.
13. **Voges–Proskauer test.** The Voges–Proskauer test detects acetylmethylcarbinol (acetoin), an intermediate product in the butene glycol pathway of glucose fermentation.

PART V

# PATHOGENIC BACTERIA

## CHAPTER 23

*Pseudomonas* and Other Opportunistic Gram-negative Bacilli

## CHAPTER 24

*Haemophilus* and *Bordetella*

## CHAPTER 25

*Mycoplasma* and *Ureaplasma*

## CHAPTER 26

*Legionella*

## CHAPTER 27

**Spirochetes**

## CHAPTER 28

**Mycobacteria**

## CHAPTER 29

*Actinomyces* and *Nocardia*

## CHAPTER 30

*Chlamydia*

## CHAPTER 31

*Rickettsia, Coxiella, Ehrlichia,* and *Bartonella*

## CHAPTER 32

**Plague and Other Bacterial Zoonotic Diseases**

CHAPTER 16

# Staphylococci

KENNETH J. RYAN

Members of the genus *Staphylococcus* (staphylococci) are Gram-positive cocci that tend to be arranged in grape-like clusters. Worldwide, *Staphylococcus aureus* is one of the most common and virulent causes of acute purulent infections. Other species are common in the skin flora but produce lower grade disease, typically in association with some abridgment of the host defenses such as an indwelling catheter.

## STAPHYLOCCOCI: GROUP CHARACTERISTICS

Although staphylococci have a marked tendency to form clusters (from the Greek staphyle, bunch of grapes), some single cells, pairs, and short chains are also seen. Staphylococci have a typical Gram-positive cell wall structure. Like all medically important cocci, they are nonflagellate, nonmotile, and non-spore-forming. Staphylococci grow best aerobically but are facultatively anaerobic. In contrast to streptococci, staphylococci produce catalase. More than one dozen species of staphylococci colonize humans; of these, three are of major medical importance: *S. aureus, S. epidermidis,* and *S. saprophyticus* (Table 16–1). The ability of *S. aureus* to form coagulase separates it from the other, less virulent species.

Staphylococci form clusters and are catalase positive

Coagulase distinguishes *S. aureus* from other species

## *Staphylococcus aureus*

### BACTERIOLOGY

#### MORPHOLOGY AND STRUCTURE

In growing cultures, the cells of *S. aureus* are uniformly Gram-positive and regular in size, fitting together in clusters with the precision of pool balls. In older cultures, in resolving lesions, and in the presence of some antibiotics, the cells often become more variable in size, and many lose their Gram positivity.

The cell wall of *S. aureus* consists of a typical Gram-positive peptidoglycan (see Chapter 2) interspersed with molecules of a ribitol-teichoic acid, which is antigenic and

TABLE 16–1

**Features of Human Staphylococci**

| | | | Pathogenic Features | | |
|---|---|---|---|---|---|
| Species | Coagulase | Common Habitat | Catheter Colonization | Furuncles | Exotoxin Production |
| *S. aureus* | + | Anterior nares, perineum | + | + | +[b] |
| *S. epidermidis* | − | Anterior nares, skin | +[a] | − | − |
| *S. saprophyticus*[c] | − | Urinary tract | + | − | − |
| Others | − | Various | +[a] | − | − |

[a] Some strains produce surface slime.
[b] Including exfoliatin pyrogenic and toxin superantigens.
[c] Species statistically associated with urinary infection in young women.

Protein A binds Fc portion of IgG

relatively specific for *S. aureus*. In most strains, the peptidoglycan of the cell wall is overlaid with surface proteins; one protein, protein A, is unique in that it binds the Fc portion of IgG molecules, leaving the antigen-reacting Fab portion directed externally. This phenomenon has been exploited in test systems for detecting free antigens (see Chapter 15). It probably contributes to the virulence of *S. aureus* by interfering with opsonization.

## CHARACTERISTICS FOR IDENTIFICATION AND SUBTYPING

Colonies are white or golden and hemolytic

After overnight incubation on blood agar, *S. aureus* produces white colonies that tend to turn a buff-golden color with time, which is the basis of the species epithet *aureus* (golden). Most, but not all, strains show a rim of clear β-hemolysis surrounding the colony.

Coagulase produces a fibrin clot

Slide clumping factor correlates with coagulase

The most important test used to distinguish *S. aureus* from other staphylococci is the production of **coagulase,** which nonenzymatically binds to prothrombin, forming a complex that initiates the polymerization of fibrin. It is demonstrated by incubating staphylococci in plasma; this produces a fibrin clot within hours. A dense emulsion of *S. aureus* cells in water also clumps immediately on mixing with plasma due to direct binding of fibrinogen to a factor on the cell surface. This is the basis of a quick laboratory test called the slide clumping test, which has a high correlation with coagulase (95%). Commercial agglutination tests that correlate well with the coagulase test are also used.

Bacteriophage typing defines fingerprints for epidemiologic investigations

*S. aureus* isolates can be organized into broad groups, and individual strains can be "fingerprinted" for epidemiologic purposes by using bacteriophage typing. This procedure depends on differing susceptibilities of the organism to lysis by bacteriophages derived from lysogenic strains of *S. aureus.* Suspensions of the phages are dropped onto a plate seeded with the staphylococcal strain to be tested, and the plates are incubated. Lysis in the area of a drop indicates susceptibility to that phage (Fig 16–1). The phage type is simply a listing of the phages that gave a positive reaction (eg, 52/52A/80/81). Phage typing is a specialized procedure performed only in a few reference laboratories.

## TOXINS AND BIOLOGICALLY ACTIVE EXTRACELLULAR ENZYMES

### α-Toxin

α-Toxin inserts in lipid bilayer to form transmembrane pores

α-Toxin is a protein secreted by almost all strains of *S. aureus* but not by coagulase-negative staphylococci. It lyses cytoplasmic membranes by direct insertion into the lipid bilayer to form transmembrane pores (Fig 16–2). The resultant egress of vital molecules leads to cell death. This action is similar to other biologically active cytolysins such as streptolysin O (see Chapter 17), complement, and the effector proteins of cytotoxic T lymphocytes.

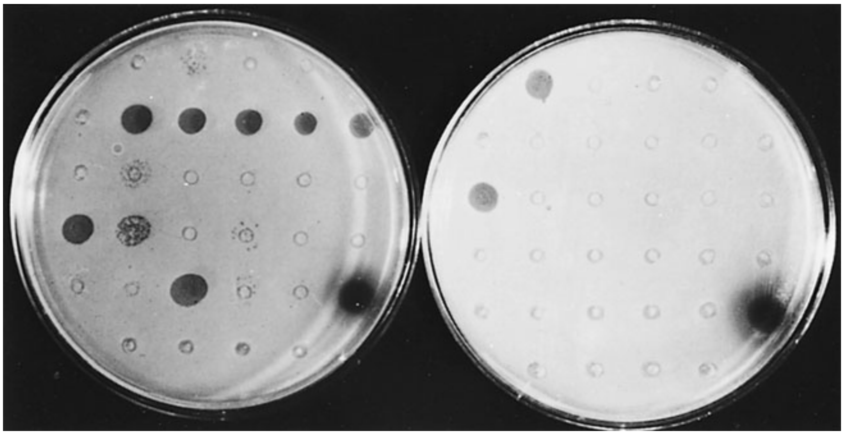

FIGURE 16–1
Bacteriophage typing of two strains of *Staphylococcus aureus:* results after overnight incubation. Lysis is indicated by absence of growth at the site of deposition of individual phages to which the strain is susceptible. The test shows that the two strains are not of common origin.

## Exfoliatin

Exfoliatin causes intercellular splitting of the epidermis between the stratum spinosum and stratum granulosum, presumably by disruption of intercellular junctions. Two antigenic variants of exfoliatin are antigenic in humans, and circulating antibody confers immunity to their effects.

Exfoliatin splits intercellular junctions

## Pyrogenic Toxin Superantigens

The pyrogenic toxin superantigens (PTSAgs) are a family of secreted proteins able to stimulate systemic effects due to absorption from the site where they are produced by multiplying staphylococci. An individual strain may produce one or more toxins but less than 10% of *S. aureus* strains produce any PTSAg. These toxins share physiochemical and biologic activity similarities with each other and PTSAgs produced by group A streptococci (see Chapter 17). As superantigens they are strongly mitogenic for T cells and do not require proteolytic processing prior to binding with class II major histocompatibility complex (MHC) molecules on antigen-presenting cells. They interact with class II MHC molecules outside the antigenic peptide groove, and are specific for the V$\beta$ region of the T-cell receptor. Thus, T cells with the appropriate V$\beta$ element may be directly activated by the toxin. This stimulates both T cells and macrophages to release massive amounts of cytokines, particularly tumor necrosis factor-$\alpha$ and interleukin-1. Other activities of these toxins are pyrogenicity and enhanced susceptibility to the lethal effects of endotoxin.

PTSAgs of group A streptococci are similar

PTSAgs bind MHC II without processing

Superantigens cause massive cytokine release

### *Staphylococcal Enterotoxins*

The ability of *S. aureus* enterotoxins to stimulate gastrointestinal symptoms (primarily vomiting) in humans and animals has long been known. There are several antigenically

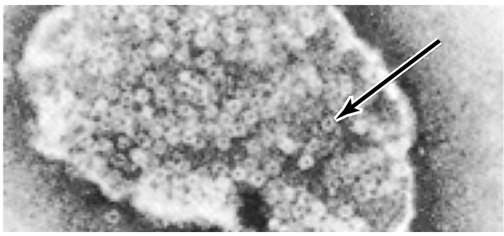

FIGURE 16–2
*Staphylococcus aureus* alpha toxin. A fragment of a rabbit erythrocyte lysed with alpha toxin is shown. Note the ring-shaped pores in the membrane created by insertion of the toxin. (From Bhadki S, Tranum-Jensen J. Alpha toxin of *Staphylococcus aureus. Microbiol Rev* 1991;55:733–751, with permission.)

Once formed, enterotoxins are stable to boiling and digestive enzymes

Vomiting is stimulated by neural mechanism

distinct low-molecular-weight proteins in this class (eg, enterotoxin A, B, C), some of which are encoded by temperate bacteriophages. Once formed, these toxins are quite stable, retaining activity even after boiling or exposure to gastric and jejunal enzymes. In addition to superantigen-mediated actions, they appear to act directly on neural receptors in the upper gastrointestinal tract, leading to stimulation of the vomiting center in the brain.

### *Toxic Shock Syndrome Toxin*

TSST-1 has superantigen and direct effects

Toxic shock syndrome toxin-1 (TSST-1), the major cause of staphylococcal toxic shock syndrome, shares many properties with the staphylococcal enterotoxins and was, in fact, confused with one of them during the course of its discovery. It can stimulate the release of cytokines through the superantigen mechanism, but may also have direct toxic effects on endothelial cells. The latter action may lead to capillary leakage, hypotension, and shock.

## STAPHYLOCOCCAL DISEASE

CLINICAL CAPSULE

Infections produced by *S. aureus* are typified by acute, aggressive, locally destructive purulent lesions. The most familiar of these is the common boil, a painful lump in the skin that has a necrotic center and fibrous reactive shell. Infections in organs other than the skin such as the lung, kidney, or bone are also focal and destructive but have greater potential for extension within the organ and beyond to the blood and other organs. Such infections typically produce high fever, systemic toxicity, and may be fatal in only a few days. A subgroup of *S. aureus* infections has manifestations produced by secreted toxins that contribute to the primary infection. Symptoms include diarrhea, rash, skin desquamation, or multiorgan effects as in staphylococcal toxic shock syndrome (TSS). Ingestion of preformed staphylococcal enterotoxin causes a form of food poisoning in which vomiting begins in only a few hours.

### EPIDEMIOLOGY

Anterior nares colonization is common

Strains with increased virulence cannot be distinguished

The basic human habitat of *S. aureus* is the anterior nares. About 30% of individuals carry the organism in this site at any given time, and rates among hospital personnel and patients may be much higher. Some nasal carriers and individuals with colonization at other sites such as the perineum may disseminate the organism extensively with desquamated epithelial cells, thus constituting a source of infection to others. The central problem in understanding the link between colonization and disease is that although we know some strains clearly have enhanced potential to produce disease, we have no way to predict which they are. Bacteriophage typing allows tracking of strains during an outbreak but by itself allows no conclusions about virulence.

Community infections are endogenous

*S. aureus* survives drying

Most *S. aureus* infections acquired in the community are autoinfections with strains that the subject has been carrying in the anterior nares, on the skin, or both. Community outbreaks are usually associated with poor hygiene and fomite transmission from individual to individual. Unlike many pathogenic vegetative organisms, *S. aureus* can survive long periods of drying; for example, recurrent skin infections can result from use of clothing contaminated with pus from a previous infection.

Hospital spread is on the hands of medical personnel

Hospital outbreaks caused by a single strain of *S. aureus* most commonly involve patients who have undergone surgical or other invasive procedures. The source of the outbreak may be a patient with an overt or inapparent staphylococcal infection (eg, decubitus ulcer) that is then spread directly to other patients on the hands of hospital personnel. A nasal or perineal carrier among medical, nursing, or other hospital personnel may also be the source of an outbreak, especially if carriage is heavy and numerous organisms are disseminated. The most hazardous source is a medical attendant who works despite having a staphylococcal lesion such as a boil. Hospital outbreaks of *S. aureus* infection can be self-perpetuating: infected patients and those who attend them frequently become carriers, and the total environmental load of the causative staphylococcus is increased. Bacteriophage typing and patterns of resistance to antimicrobics (antibiograms) are used as epidemiologic tools to detect carriers who may have initiated or contributed to continuation of the outbreak. The principles of control of epidemics in general and of hospital outbreaks are described in Chapters 12 and 72.

Outbreaks involve nasal carrier or worker with lesion

Phage typing and antibiograms are useful tools

Staphylococcal food poisoning has been an unhappy and embarrassing sequel to innumerable group picnics and wedding receptions in which gastronomic delicacies have been exposed to temperatures that allow bacterial multiplication. Characteristically, the food is moist and rich (eg, potato salad, creamy dishes). The food becomes contaminated by a preparer who is a nasal carrier or has a staphylococcal lesion. If the food is inadequately refrigerated, the staphylococci multiply and produce enterotoxin in the food. Because of the heat resistance of the toxin, toxicity persists even if the food is boiled before eating.

Enterotoxin is produced in rich foods before they are ingested

## PATHOGENESIS

### Primary Infection

The initial stages of colonization by *S. aureus* are mediated by a number of surface proteins, each of which binds to host elements in or covering tissues, body fluids, or foreign bodies such as catheters. Proteins that bind to fibronectin, fibrinogen, and collagen have been discovered, and others are under investigation. Mechanisms for bacterial extension beyond the surface are not clearly understood. Of the many potential virulence factors produced by *S. aureus,* none can be assigned the single or even primary role contributing to the ability of the bacteria to multiply and cause progressive lesions in tissues. In fact, *S. aureus* is generally of quite low infectivity unless trauma, foreign matter, or other local conditions provide access for initiation of infection. Experimentally, intradermal injection of up to $10^6$ organisms is required to initiate a local lesion unless a suture or talcum powder is added with the bacteria.

Surface proteins bind to tissue elements such as fibronectin

Trauma and foreign matter lower infecting dose

Once beyond the mucosal or skin barrier, any mechanism that protects the organisms from phagocytosis may allow multiplication to continue long enough for products such as $\alpha$-toxin to initiate local injury. One factor known to interfere with phagocytosis is surface protein A. Its binding to the Fc portion of IgG may compete with phagocytic cells for available IgG–Fc sites, thus effectively diminishing opsonization. Production of coagulase can retard migration of phagocytes to the site of infection, and even phagocytosed *S. aureus* may resist lysosomal killing. The acute inflammatory response continues, and the developing lesion has a marked tendency for localization, perhaps due to the fibrotic reaction to the $\alpha$-toxin–mediated injury to host cells.

Resistance to phagocytosis allows $\alpha$-toxin production

Protein A competes for IgG–Fc sites

The fate of the lesion depends on the ability of the host to localize the process, which differs depending on the tissue involved. In the skin, spontaneous resolution of the boil by granulation and fibrosis is the rule. In the lung, kidney, bone, and other organs, the process may continue to spread with satellite foci and involvement of broad areas. In all instances the action of the cytotoxins is highly destructive, creating cavities and massive necrosis with little respect to anatomic boundaries. In the worst cases, the staphylococci are not contained, spreading to the bloodstream and distant organs. Circulating staphylococci may also shed cell wall peptidoglycans, producing massive complement activation, leukopenia, thrombocytopenia, and a clinical syndrome of septic shock.

Destruction and spread are prominent

Peptidoglycan fragments may trigger shock

### Toxin-mediated Disease

If the strain of *S. aureus* causing any of the effects described above also produces one or more of the exotoxins, those actions are added to those of the primary infection. The primary infection serves as a site for absorption of the toxin and need not be extensive or even clinically apparent for the toxic action to occur. In staphylococcal food poisoning, there is no infection at all. The contaminating bacteria produce pyrogenic exotoxin in the food that can initiate its enterotoxic action on the intestine within hours of its ingestion.

Preformed enterotoxin acts within hours

Exfoliative toxin causes blisters or scalded skin syndrome

The in vivo production of toxin takes at least a few days and may exert its effect locally or systemically. Exfoliative toxin-producing strains cause blisterlike separation of the epidermis by their action on intercellular junctions, which is most commonly localized to the site of skin infection. In staphylococcal scalded skin syndrome, absorbed toxin causes extensive epithelial desquamation at sites remote from the primary infection.

TSST-1–producing strain must colonize vagina

Menstruation and tampons enhance local toxin production

In staphylococcal TSS, the pyrogenic exotoxin TSST-1 is produced during the course of a staphylococcal infection with systemic disease as a result of absorption of toxin from the local site. Menstruation-associated TSS requires a combination of improbable events. Less than 5% of women carry *S. aureus* in their vaginal flora, and only one in five of these staphylococci have the potential to produce TSST-1. In the presence of such a strain, the combination of menstruation and high-absorbency tampon usage appear to provide growth conditions that enhance the production of TSST-1. Toxin absorbed from the vagina can then circulate to produce superantigen-mediated cytokine release and direct effects on the vasculature (Fig 16–3).

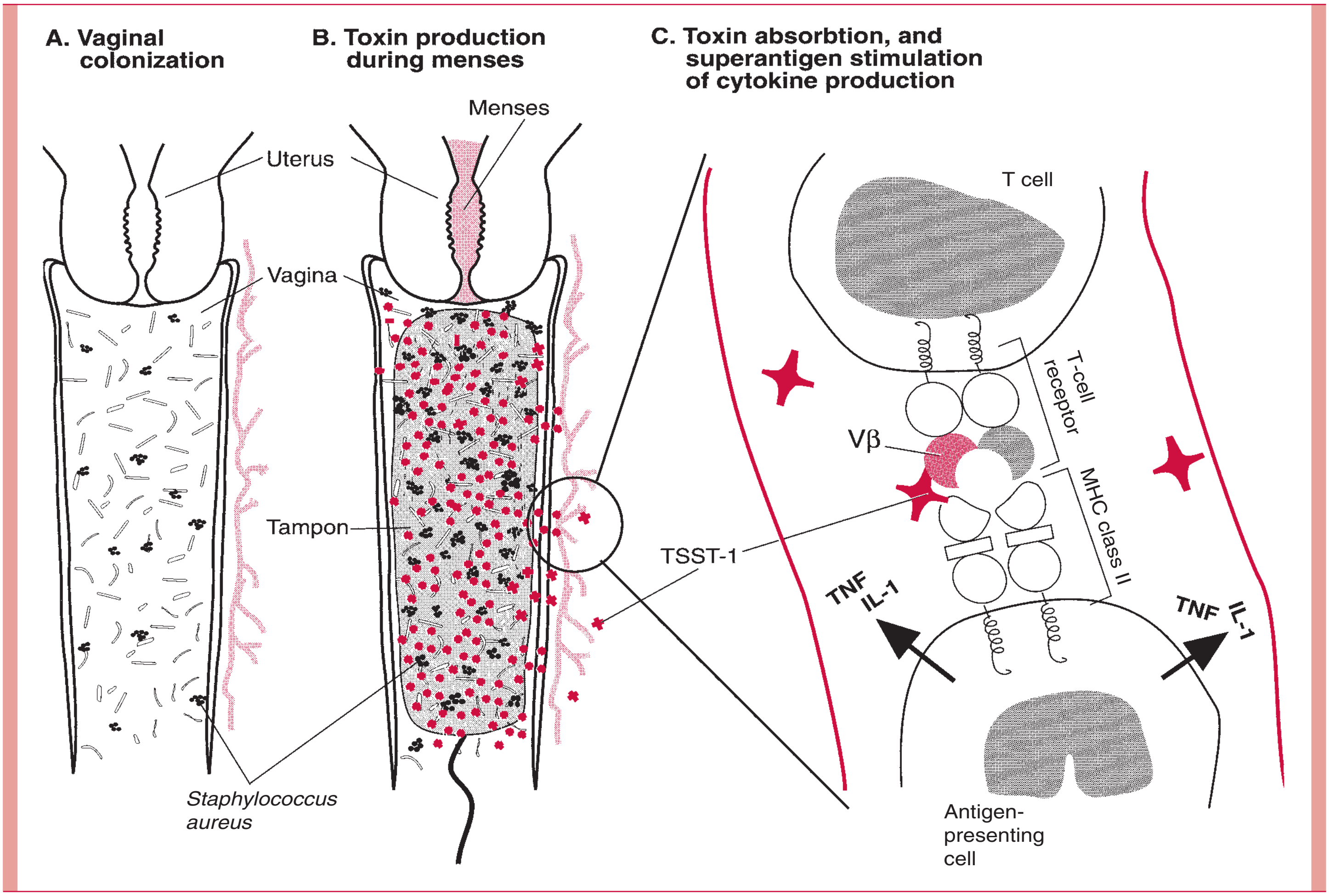


FIGURE 16–3

Pathogenesis of staphylococcal toxic shock syndrome. **A.** The vagina is colonized with normal flora and a strain of *Staphylococcus aureus* containing the TSST-1 gene. **B.** The conditions with tampon usage facilitate growth of the *S. aureus* and TSST-1 production. **C.** The toxin is absorbed from the vagina and circulates. The systemic effects may be due to the direct effect of the toxin or via cytokines released by the superantigen mechanism. The toxin is shown binding directly with the V$\beta$ portion of the T-cell receptor and the class II major histocompatibility complex (MHC) receptor. This V$\beta$ stimulation signals the production of cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF).

Some cases of full-blown staphylococcal TSS are associated with strains that do not produce TSST-1. This is particularly true of nonmenstrual cases. Other PTSAgs have been detected in these strains and have been shown to produce experimental toxic shock. TSS may be the result of in vivo production of any of the staphylococcal pyrogenic exotoxins, with TSST-1 simply the most common offender. The mechanisms by which the pyrogenic exotoxins produce the multiple renal, cutaneous, intestinal, and cardiovascular manifestations of TSS are not known.

Nonmenstrual TSS cases may have any PTSAg-producing strain

## IMMUNITY

The natural history of staphylococcal infections indicates that immunity is of short duration and incomplete. Chronic furunculosis, for example, can recur over many years. The relative roles of humoral and cellular immune mechanisms are uncertain, and attempts to induce immunity artificially with various staphylococcal products have been disappointing at best. In menstruation-associated TSS, many patients have low or absent antibody levels to TSST-1 and often fail to mount a significant antibody response during the disease. Repeated attacks have been recorded, suggesting a genetic predisposition.

Immunity is poorly understood

Relapsing infections show little evidence of immunity

# STAPHYLOCOCCAL INFECTIONS: CLINICAL ASPECTS

## MANIFESTATIONS: PRIMARY INFECTION

### Furuncle and Carbuncle

The furuncle or boil is a superficial skin infection that develops in a hair follicle, sebaceous gland, or sweat gland. Blockage of the gland duct with inspissation of its contents causes predisposition to infection. Furunculosis is often a complication of acne vulgaris. Infection at the base of the eyelash gives rise to the common stye. The infected patient is often a carrier of the offending *Staphylococcus,* usually in the anterior nares. The course of the infection is usually benign, and the infection resolves upon spontaneous drainage of pus. No surgical or antimicrobic treatment is needed. Infection can spread from a furuncle with the development of one or more abscesses in adjacent subcutaneous tissues. This lesion, known as a carbuncle, occurs most often on the back of the neck but may involve other skin sites. Carbuncles are serious lesions that may result in bloodstream invasion (bacteremia).

Focal lesions drain spontaneously

Boils develop in hair follicles

Multiple boils become a carbuncle

### Chronic Furunculosis

Some individuals are subject to chronic furunculosis, in which repeated attacks of boils are caused by the same strain of *S. aureus.* There is little, if any, evidence of acquired immunity to the disease; indeed, delayed-type hypersensitivity to staphylococcal products appears responsible for much of the inflammation and necrosis that develops. Chronic staphylococcal disease may be associated with factors that depress host immunity, especially in patients with diabetes or congenital defects of polymorphonuclear leukocyte function. However, in most instances, predisposing disease other than acne is not present.

Links to immune dysfunction are limited

### Impetigo

*S. aureus* is most often seen as a secondary invader in group A streptococcal pustular impetigo (see Chapter 17), but it can produce the skin pustules of impetigo on its own. Strains of *S. aureus* that produce exfoliatin cause a characteristic form called bullous impetigo, characterized by large blisters containing many staphylococci in the superficial layers of the skin. Bullous impetigo can be considered a localized form of scalded skin syndrome.

Exfoliatin-producing strains cause bullous impetigo

## Deep Lesions

Acute osteomyelitis is primarily a *S. aureus* disease

Pneumonia and deep tissue lesions are highly destructive

Bacteremic spread and endocarditis are most common in drug abusers

*S. aureus* can cause a wide variety of infections of deep tissues by bacteremic spread from a skin lesion that may be unnoticed. These include infections of bones, joints, deep organs, and soft tissues, including surgical wounds. More than 90% of the cases of acute osteomyelitis in children are caused by *S. aureus.* Staphylococcal pneumonia is typically secondary to some other insult to the lung, such as influenza, aspiration, or pulmonary edema. At deep sites the organism has the same tendency to produce localized, destructive abscesses that it does in the skin. All too often the containment is less effective, and spread with multiple metastatic lesions occurs. Bacteremia and endocarditis can develop. All are serious infections that constitute acute medical emergencies. In all of these situations, diabetes, leukocyte defects, or general reduction of host defenses by alcoholism, malignancy, old age, or steroid or cytotoxic therapy can be predisposing factors. Severe *S. aureus* infections, including endocarditis, are particularly common in drug abusers using injection methods.

# MANIFESTATIONS CAUSED BY STAPHYLOCOCCAL TOXINS

## Scalded Skin Syndrome

Widespread desquamation in neonates is caused by exfoliatin-producing strains

Staphylococcal scalded skin syndrome results from the production of exfoliatin in a staphylococcal lesion, which can be quite minor (eg, conjunctivitis). Erythema and intraepidermal desquamation takes place at remote sites from which *S. aureus* cannot be isolated (Fig 16–4). The disease is most common in neonates and children less than 5 years of age. The face, axilla, and groin tend to be affected first, but the erythema, bullous formation, and subsequent desquamation of epithelial sheets can spread to all parts of the body. The disease occasionally occurs in adults, particularly those who are immunocompromised. Milder versions of what is probably the same disease are staphylococcal scarlet fever, in which erythema occurs without desquamation, and bullous impetigo, in which local desquamation occurs.

## Toxic Shock Syndrome

Fever, vomiting, diarrhea, and muscle pain are early findings

Shock, renal and hepatic injury may follow

Toxic shock syndrome (TSS) was first described in children but came to public attention during the early 1980s, when hundreds of cases were reported in young women using intravaginal tampons. The disease is characterized by high fever, vomiting, diarrhea, sore throat, and muscle pain. Within 48 hours, it may progress to severe shock with evidence of renal and hepatic damage. A skin rash may develop, followed by desquamation at a deeper level than in scalded skin syndrome. Blood cultures are usually negative. The outbreak receded with the withdrawal of certain brands of highly absorbent tampons.

FIGURE 16–4
Staphylococcal scalded skin syndrome in a neonate. The focal staphylococcal infection was a breast abscess in the infant.

### Staphylococcal Food Poisoning

Ingestion of staphylococcal enterotoxin contaminated food results in acute vomiting and diarrhea within 1 to 5 hours. There is prostration, but usually no fever. Recovery is rapid, except sometimes in the elderly and in those with another disease.

Vomiting is prominent without fever

## DIAGNOSIS

Laboratory procedures to assist in diagnosis of staphylococcal infections are quite simple. Most acute, untreated lesions contain numerous polymorphonuclear leukocytes and large numbers of Gram-positive cocci in clusters. Staphylococci grow overnight on blood agar incubated aerobically. Catalase and coagulase tests performed directly from the colonies are sufficient for identification. Antibiotic susceptibility tests are indicated because of the emerging resistance of *S. aureus* to multiple antimicrobics, particularly methicillin and vancomycin.

Gram stain and culture are primary diagnostic methods

Deep staphylococcal infections such as osteomyelitis or deep abscesses present special diagnostic problems when the lesion cannot be directly aspirated or surgically sampled. Blood cultures are usually positive in conditions such as acute staphylococcal arthritis, osteomyelitis, and endocarditis but less often in localized infection such as deep abscesses.

Aspirates and blood cultures are necessary for deep infections

## TREATMENT

Most boils and superficial staphylococcal abscesses resolve spontaneously without antimicrobial therapy. Those that are more extensive, deeper, or in vital organs require a combination of surgical drainage and antimicrobics for optimal outcome. Penicillins and cephalosporins are active against *S. aureus* cell wall peptidoglycan and vary in their susceptibility to inactivation by staphylococcal $\beta$-lactamases. Although penicillin G is the treatment of choice for susceptible strains, the penicillinase-resistant penicillins (methicillin, nafcillin, oxacillin) and first-generation cephalosporins are more commonly used because of resistance. For strains resistant to these agents or patients with $\beta$-lactam hypersensitivity, the alternatives are vancomycin, clindamycin, or erythromycin. Synergy between cell wall–active antibiotics and the aminoglycosides is present when the staphylococcus is sensitive to both types of agents. Such combinations are often used in severe systemic infections when effective and rapid bactericidal action is needed, particularly in compromised hosts.

Superficial lesions resolve spontaneously

Penicillinase-resistant $\beta$-lactams are used pending susceptibility tests

## ANTIMICROBIAL RESISTANCE

When penicillin was introduced to the general public following World War II, virtually all strains of *S. aureus* were highly susceptible. Since then, the selection of preexisting strains able to produce a penicillinase has shifted these proportions to the point at which 80 to 90% of isolates are now penicillin resistant. The penicillinase is encoded by plasmid genes and acts by opening the $\beta$-lactam ring, making the drug unable to bind with its target.

Most strains of *S. aureus* are now penicillin resistant

Penicillinase production is plasmid mediated

Alterations in the $\beta$-lactam target, the peptidoglycan transpeptidases (often called penicillin-binding proteins, or PBPs), is the basis for resistance to methicillin. These methicillin-resistant *S. aureus* (MRSA) strains are also resistant to the other penicillinase-resistant penicillins such as oxacillin. The most common mechanism is the acquisition of a gene for a new transpeptidase, which has reduced affinity for $\beta$-lactam antibiotics, but is still able to carry out its enzymatic function of cross-linking peptidoglycan.

Methicillin-resistant strains produce new PBP

The frequency of MRSA has great geographic variation. Most American hospitals report MRSA rates of 5 to 25%, but outbreaks are increasing and resistance rates over 50% have been reported in other countries. There are some problems in detecting MRSA; resistant cells may represent only a small portion of the total population (heteroresistance). Tests are generally performed with methicillin or oxacillin under technical conditions that facilitate detection of the resistant subpopulation, and the results extrapolated to other relevant agents. For example, oxacillin resistance is considered proof of resistance to methicillin, nafcillin, dicloxacillin, and all cephalosporins. Vancomycin is often used to treat

MRSA rates are variable but increasing

MRSA detection requires special conditions

Vancomycin use for MRSA is threatened

serious infections with MRSA. The recent emergence of *S. aureus* with decreased susceptibility to vancomycin is of great concern, these strains are still very rare.

## PREVENTION

Antistaphylococcal soaps block infection

Elimination of nasal carriage is difficult

In patients subject to recurrent infection, such as chronic furunculosis, preventive measures are aimed at controlling reinfection and, if possible, eliminating the carrier state. Clothes and bedding that may cause reinfection should be washed at a sufficiently high temperature to destroy staphylococci (70°C or higher) or dry-cleaned. In adults, the use of chlorhexidine or hexachlorophene soaps in showering and washing increases the bactericidal activity of the skin (see Chapter 11). In such individuals, or persons found to be a source of an outbreak, anterior nasal carriage can be reduced and often eliminated by the combination of nasal creams containing topical antimicrobics (eg, mupirocin, neomycin, and bacitracin) and oral therapy with antimicrobics that are concentrated within phagocytes and nasal secretions (eg, rifampin or ciprofloxacin). Attempts to reduce nasal carriage more generally among medical personnel in an institution are usually fruitless and encourage replacement of susceptible strains with multiresistant ones.

Chemoprophylaxis during high-risk surgery is effective

Chemoprophylaxis is effective in surgical procedures such as hip and cardiac valve replacements, in which infection with staphylococci can have devastating consequences. Methicillin, a cephalosporin, or vancomycin given during and shortly after surgery may reduce the chance for intraoperative infection while minimizing the risk for superinfection associated with longer periods of antibiotic administration.

# *Coagulase-Negative Staphylococci*

Common colonizers of the skin

Commonly colonize implanted medical devices

*S. epidermidis* and a number of other species of coagulase-negative staphylococci are normal commensals of the skin, anterior nares, and ear canals of humans. Their large numbers and ubiquitous distribution result in frequent contamination of specimens collected from or through the skin, making these organisms among the most frequently isolated in the clinical laboratory. In the past, they were rarely the cause of significant infections, but with the increasing use of implanted catheters and prosthetic devices, they have emerged as important agents of hospital-acquired infections. Immunosuppressed or neutropenic patients and premature infants have been particularly affected.

Polysaccharide slime production enhances attachment and survival

Organisms may contaminate prosthetic devices during implantation, seed the device during a subsequent bacteremia, or gain access to the lumina of shunts and catheters when they are temporarily disconnected or manipulated. The outcome of the bacterial contamination is determined by the ability of the microbe to attach to the surface of the foreign body and to multiply there. Initial adherence is facilitated by the hydrophobic nature of the synthetic polymers used in medical devices and the natural hydrophobic nature of many coagulase-negative staphylococci. Following attachment, some strains produce a viscous extracellular polysaccharide **slime** or biofilm. This biofilm provides additional adhesion, completely covers the bacteria, and serves as a mechanical barrier to antimicrobial agents and host defense mechanisms; it is also believed to enhance nutrition of the microbes by functioning as an ion-exchange resin. Strains able to produce the polysaccharide biofilm are more likely to colonize intravenous catheters but have no known advantage in adherence to human tissues such as heart valves. The resistance of many coagulase-negative staphylococci to multiple antimicrobic agents contributes further to their persistence in the body. Infections are generally low grade, but unless controlled, they can proceed to serious tissue damage or a fatal outcome.

Most common skin contaminant in cultures

The interpretation of cultures that grow coagulase-negative staphylococci is fraught with difficulty. In most cases, the finding is attributable to skin contamination, although it can indicate infection when a patient has implanted devices, or has defenses that are otherwise compromised. The presence of at least moderate numbers of organisms or the repeated isolation of a strain with the same antibiogram argues for infection over skin contamination. There is no phage-typing system for coagulase-negative staphylococci but a number of molecular procedures (see Chapter 15) have been used to compare isolates for epidemiologic purposes.

Repeated positives suggest infection

Most coagulase-negative staphylococci now encountered are resistant to penicillin, and many are also methicillin resistant. Resistance to multiple antimicrobics usually active against Gram-positive cocci, including vancomycin, is more common than with *S. aureus.* Eradication of coagulase-negative staphylococci from prosthetic devices and associated tissues with chemotherapy alone is very difficult unless the device is also removed.

Multiple antimicrobic resistance is common

## ADDITIONAL READING

Chambers HF. Methicillin resistance in staphylococci: Molecular and biochemical basis and clinical implications. *Clin Microbiol Rev* 1997;10:781–791. The complex topic of staphylococcal heteroresistance and its detection is clearly explained in only seven pages. A discussion of alternate treatment strategies is also included.

Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus. Clin Microbiol Rev* 2000;13:16–34. The structural biology and the role of the pyrogenic exotoxins in food poisoning and TSS are carefully but concisely explained. The locally acting toxins are also discussed.

Elek SD, Conan PE. The virulence of *Staphylococcus pyogenes* for man. A study of the problems of wound infections. *Br J Exp Pathol* 1957;38:573–586. A classic study of the factors influencing the development of staphylococcal wound infections in humans.

Lowry FD. *Staphylococcus aureus* infections. *N Engl J Med* 1998;339:520–532. A review that considers the epidemiologic, clinical, therapeutic, and pathogenesis of *S. aureus* infection. The pathogenesis discussion is particularly well-illustrated.

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CHAPTER 17

# Streptococci and Enterococci

KENNETH J. RYAN

Bacteria of the genus *Streptococcus* are Gram-positive cocci arranged in chains that form a significant portion of the indigenous microflora of the oropharynx. In addition to relatively harmless species, the genus includes three of the most important pathogens of humans. One is *S. pyogenes,* the cause of "strep throat," which can lead to rheumatic fever and heart disease; the ability of some strains to cause catastrophic deep tissue infections recently led British tabloids to give them the gory label "flesh-eating bacteria." Second is *S. agalactiae,* the most frequent cause of sepsis in newborns. Third is *S. pneumoniae,* a leading cause of pneumonia and meningitis in persons of all ages.

## STREPTOCOCCI

## *Group Characteristics*

### MORPHOLOGY

Streptococci stain readily with common dyes, demonstrating coccal cells that are generally smaller and more ovoid in shape than staphylococci. They are usually arranged in chains with oval cells touching end to end, because they divide in one plane and tend to remain attached. Length may vary from a single pair to continuous chains of over 30 cells, depending on the species and growth conditions. Medically important streptococci are not acid fast, do not form spores, and are nonmotile. Some members form capsules composed of polysaccharide complexes or hyaluronic acid.

Oval cells arranged in chains end to end

### CULTURAL AND BIOCHEMICAL CHARACTERISTICS

Streptococci grow best in enriched media under aerobic or anaerobic conditions (facultative). Growth of many strains is enhanced by the presence of carbon dioxide. Blood agar is preferred because it satisfies the growth requirements and also serves as an indicator for patterns of hemolysis. The colonies are small, ranging from pinpoint size to 2 mm in diameter, and they may be surrounded by a zone where the erythrocytes suspended in the agar have been hemolyzed. When this zone is clear, this state is called **$\beta$-hemolysis.**

$\beta$ hemolysis is clear

$\alpha$ hemolysis is incomplete, with greening of blood agar

Catalase negative

When the result is hazy (incomplete hemolysis), with a green discoloration of the agar, it is called **$\alpha$-hemolysis.** Streptococci are metabolically active, attacking a variety of carbohydrates, proteins, and amino acids. Glucose fermentation yields mostly lactic acid. In contrast to staphylococci, streptococci are catalase negative.

## CLASSIFICATION

Lancefield antigens are cell wall carbohydrates

Presence of Lancefield antigens defines the pyogenic streptococci

At the turn of the 20th century, a classification based on hemolysis and biochemical tests was sufficient to associate some streptococcal species with infections in humans and animals. Rebecca Lancefield, who demonstrated carbohydrate antigens in cell-wall extracts of the $\beta$-hemolytic streptococci, put this taxonomy on a sounder basis. Her studies formed a classification by serogroups (eg, A, B, C), each of which is generally correlated with an established species. Later it was discovered that some nonhemolytic streptococci had the same cell wall antigens. Over the years it has become clear that possession of one of the Lancefield antigens defines a particularly virulent segment of the streptococcal genus regardless of hemolytic patterns. These are called the **pyogenic streptococci,** and in medical circles they are now better known by their Lancefield letter than the older species name. Pediatricians instantly recognize GBS as an acronym for group B streptococcus but may be confused by use of the proper name, *Streptococcus agalactiae* (Table 17–1).

Hemolysis is a practical guide to classification

Only pyogenic streptococci are $\beta$-hemolytic

For practical purposes, the type of hemolysis and certain biochemical reactions remain valuable for the initial recognition and presumptive classification of streptococci, and as an indication of what subsequent taxonomic tests to perform. Thus, $\beta$-hemolysis indicates that the strain has one of the Lancefield group antigens, but some Lancefield positive strains or groups may be $\alpha$-hemolytic or even nonhemolytic. The streptococci will be considered as follows: (1) pyogenic streptococci (Lancefield groups); (2) pneumococci; (3) viridans and other streptococci (see Table 17–1).

### Pyogenic Streptococci

Groups A and B streptococci are most common cause of disease

Of the many Lancefield groups, the ones most frequently isolated from humans are A, B, C, F, and G. Of these, groups A (*S. pyogenes)* and B (*S. agalactiae*) are the most frequent causes of serious disease. The group D carbohydrate is found in the genus *Enterococcus,* which used to be classified among the streptococci.

### Pneumococci

Pneumococci have an antigenic polysaccharide capsule

This category contains a single species, *S. pneumoniae,* commonly called the pneumococcus. Its distinctive feature is the presence of a capsule composed of polysaccharide polymers that vary in antigenic specificity. More than 90 capsular immunotypes have been defined. Although the pneumococcal cell wall shares some common antigens with other streptococci, it does not possess any of the Lancefield group antigens. *S. pneumoniae* is $\alpha$-hemolytic.

### Viridans and Other Streptococci

Viridans and nonhemolytic species lack Lancefield antigens or capsules

Viridans streptococci are $\alpha$-hemolytic and lack both the group carbohydrate antigens of the pyogenic streptococci and the capsular polysaccharides of the pneumococcus. The term encompasses several species, including *S. salivarius* and *S. mitis*. Viridans streptococci comprise members of the normal oral flora of humans. They rarely demonstrate invasive qualities. A variety of other streptococci may be encountered that lack the features of the pyogenic streptococci or pneumococci; they would be classified with the viridans group, except that they are not $\alpha$-hemolytic. Such strains are usually assigned descriptive terms such as nonhemolytic streptococci or microaerophilic streptococci. They have been less thoroughly studied, but generally have the same biologic behavior as the viridans streptococci.

TABLE 17–1

**Classification of Streptococci and Enterococci**

| Group/Species | Common Term | Hemolysis | Major Antigens/Structures: Lancefield Cell Wall | Major Antigens/Structures: Surface Protein | Major Antigens/Structures: Capsule | Virulence Factors | Disease |
|---|---|---|---|---|---|---|---|
| **Streptococci** | | | | | | | |
| **Pyogenic** | | | | | | | |
| *Streptococcus pyogenes* | Group A strep, GAS | $\beta$ | A | M protein (80+) | Hyaluronic acid | M protein, leipoteichoic acid, streptococcal pyrogenic exotoxins, streptolysin O, streptokinase | Strep throat, impetigo, pyogenic infections, toxic shock, rheumatic fever, glomerulonephritis |
| *S. agalactiae* | Group B strep, GBS | $\beta$, – | B | – | Sialic acid (9) | Capsule | Neonatal sepsis, meningitis, pyogenic infections |
| *S. equi* | | $\beta$ | C | – | – | – | Pyogenic infections |
| *S. bovis* | | –, $\alpha$ | D | – | – | – | Pyogenic infections |
| Other species | | $\beta$, $\alpha$, – | E-W | – | – | – | Pyogenic infections |
| **Pneumococcus** | | | | | | | |
| *S. pneumoniae* | Pneumococcus | $\alpha$ | – | Choline-binding protein | Polysaccharide (90+) | Capsule, pneumolysin, neuraminidase | Pneumonia, meningitis, otitis media, pyogenic infections |
| **Viridans and Nonhemolytic** | | | | | | | |
| *S. sanguis* | | $\alpha$ | – | – | – | – | Low virulence, endocarditis |
| *S. salivarius* | | $\alpha$ | – | – | – | – | Low virulence, endocarditis |
| *S. mutans* | | $\alpha$ | – | – | | | Dental caries |
| Other species | | $\alpha$, – | – | – | – | – | Low virulence, endocarditis |
| **Enterococci** | | | | | | | |
| *Enterococcus faecalis* | Enterococcus | –, $\alpha$ | D | – | – | – | Urinary tract, pyogenic infections |
| *E. faecium* | Enterococcus | –, $\alpha$ | D | – | – | – | Urinary tract, pyogenic infections |
| Other species | | –, $\alpha$ | D, – | – | – | – | Urinary tract, pyogenic infections |

# Group A Streptococci (Streptococcus pyogenes)

## BACTERIOLOGY

### MORPHOLOGY AND GROWTH

Streptolysin O or S cause β-hemolysis

Aerobically, only S is active

Group A streptococci typically appear in purulent lesions or broth cultures as spherical or ovoid cells in chains of short to medium length (4 to 10 cells). On blood agar plates, colonies are usually compact, small, and surrounded by a 2- to 3-mm zone of β hemolysis that is easily seen and sharply demarcated. β-hemolysis is caused by either of two hemolysins, **streptolysin S** and the oxygen-labile **streptolysin O,** both of which are produced by most group A strains. Strains that lack streptolysin S are β-hemolytic only under anaerobic conditions, because the remaining streptolysin O is not active in the presence of oxygen. This feature is of practical importance, because such strains would be missed if cultures were incubated only aerobically.

### STRUCTURE

Wall contains group antigen with multiple surface molecules extending beyond

The structure of group A streptococci is illustrated in Figure 17–1. The cell wall is built on a peptidoglycan matrix that provides rigidity, as in other Gram-positive bacteria. Within this matrix lies the group carbohydrate antigen, which by definition is present in all group A

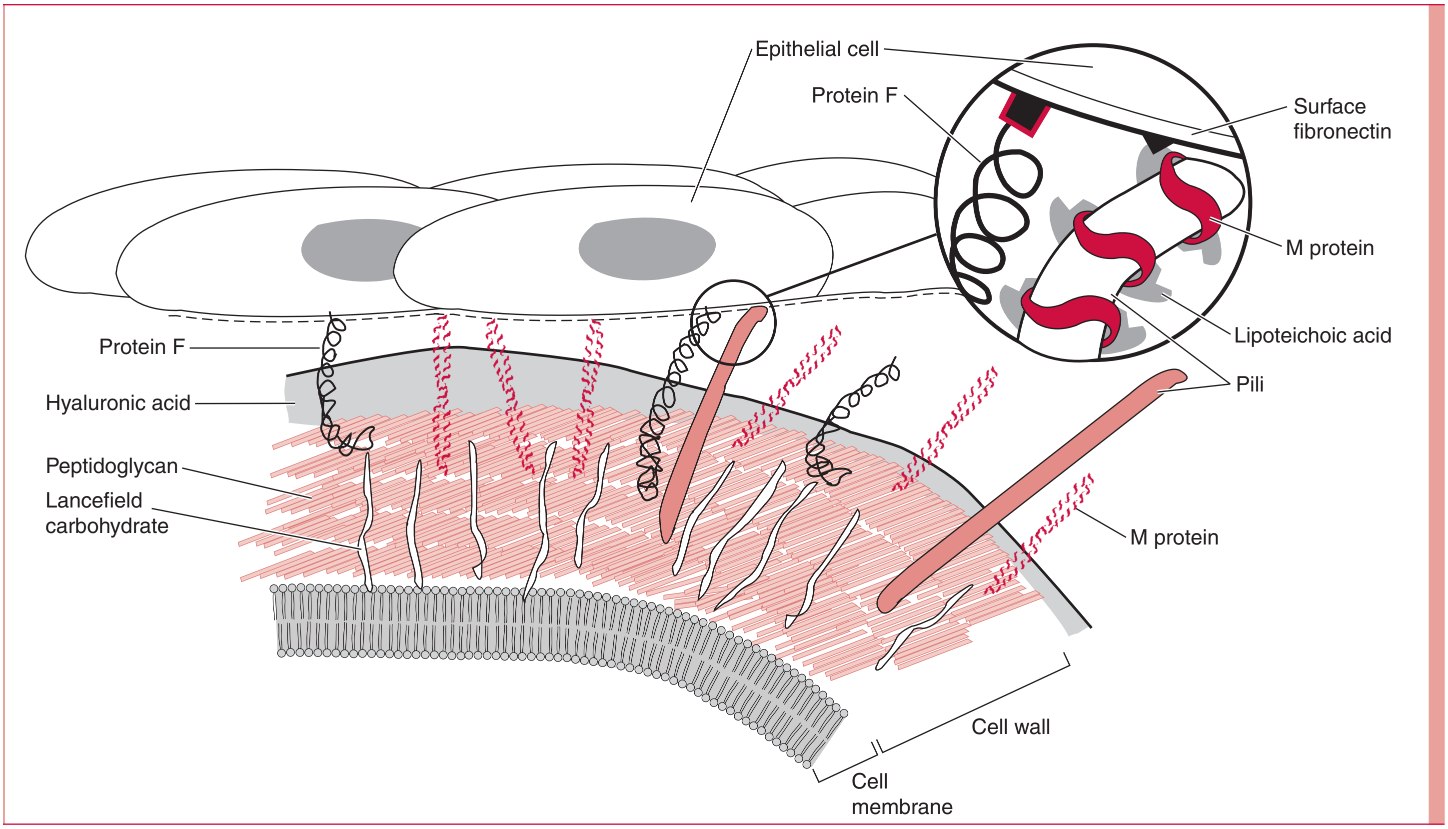


FIGURE 17–1

Antigenic structure of *S. pyogenes* and adhesion to an epithelial cell. The location of peptidoglycan and Lancefield carbohydrate antigen in the cell wall is shown in the diagram. M protein and lipoteichoic acid are associated with the cell surface and the pili. Lipoteichoic acid and protein F mediate binding to fibronectin on the host surface.

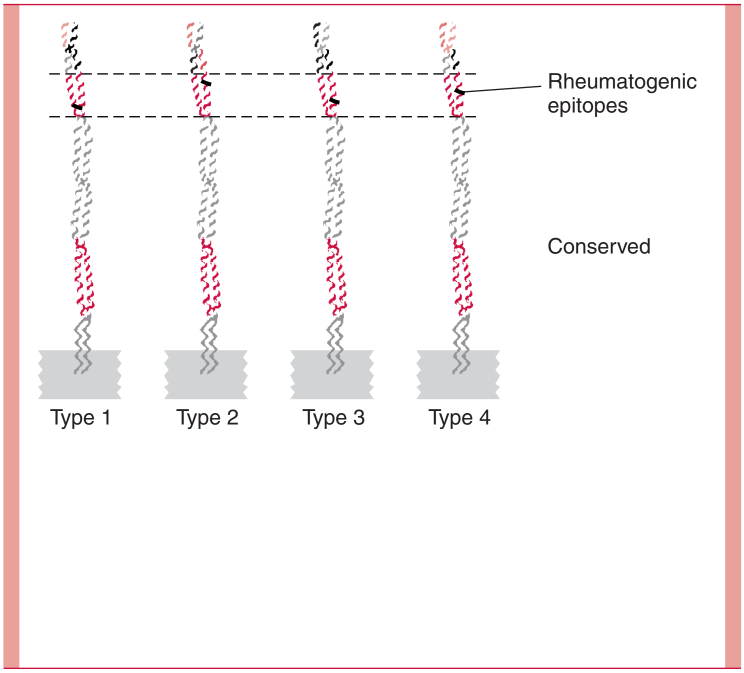


FIGURE 17–2
The coiled-coil structure of M protein is shown. The most variable parts of the molecule are oriented to the outside and provide the antiphagocytic effect and serologic specificity. The conserved potions are rooted in the cell wall. All four types contain epitopes which may stimulate the cross-reactive immune reactions seen in rheumatic fever.

streptococci. A number of other molecules such as M protein and lipoteichoic acid (LTA) are attached to the cell wall but extend beyond often in association with the hair-like pili. Group A streptococci are divided into more than 80 serotypes based on antigenic differences in the M protein. Some strains have an overlying nonantigenic hyaluronic acid capsule.

### M Protein

The M protein itself is a fibrillar coiled-coil molecule (Fig 17–2) with structural homology to myosin. Its carboxy terminus is rooted in the peptidoglycan of the cell wall, and the amino-terminal regions extend out from the surface. The specificity of the more than 80 serotypes of M protein is determined by variations in the amino sequence of the amino-terminal portion of the molecule. Because of its location, this part of the M protein is also the most available to immune surveillance. The middle part of the molecule is less variable, and some carboxy terminal regions are conserved across many M types. There is increasing evidence that some the many known biologic functions of M protein can be assigned to specific domains of the molecule. This includes both antigenicity and the capacity to bind other molecules such as fibrinogen, serum factor H, and immunoglobulins.

Coiled-coil is similar to myosin

Antigenic variation and function differ in domains of the molecule

80+ M protein serotypes exist

### Other Surface Molecules

A number of surface proteins have been described on the basis of their similarity with M protein or some unique binding capacity. Of these, a fibronectin binding **protein F** and **LTA** are both exposed on the streptococcal surface (see Fig 17–1) and may have a role in pathogenesis. An IgG binding protein has the capacity to bind the Fc portion of antibodies in much the same way as staphylococcal protein A. In principle, this could interfere with opsonization by creating a covering of antibody molecules on the streptococcal surface that are facing the "wrong way." Group A streptococci may have a **hyaluronic acid capsule,** which is a polymer containing repeating units of glucuronic acid and N-acetylglucosamine.

Protein F and LTA bind fibronectin

Hyaluronic acid capsule may be present

## BIOLOGICALLY ACTIVE EXTRACELLULAR PRODUCTS

### Streptolysin O

Streptolysin O is a general cytotoxin, lysing leukocytes, tissue cells, and platelets. The toxin inserts directly into the cell membrane of host cells, forming transmembrane pores in a manner similar to complement and staphylococcal $\alpha$-toxin (see Chapter 16). Streptolysin

Streptolysin O is pore-forming and antigenic

O is antigenic and the quantitation of antibodies against it is the basis of a standard serologic test called antistreptolysin O (ASO).

### Pyrogenic Exotoxins

SPEs are produced by some strains

SPEs are superantigens and some have enzymatic activity

The manifestations of classical **scarlet fever** have long been associated with the action of an erythrogenic toxin. This toxin is now included in a family of nine proteins called **streptococcal pyrogenic exotoxins (SPEs),** one of which is produced by approximately 10% of group A streptococci. The SPEs are identified by letters (eg, A, B, C) and are similar in structure and biological activity to the pyrogenic exotoxins produced by *Staphylococcus aureus*. They have multiple effects including fever, rash (scarlet fever), T-cell proliferation, B-lymphocyte suppression, and heightened sensitivity to endotoxin. At least some of these actions are due to cytokine release through the superantigen mechanism (see Chapter 8). SPE-B also has enzymatic activity cleaving elements of the extracellular matrix, including fibronectin and vitronectin.

### Other Extracellular Products

C5a peptidase degrades complement

Streptokinase converts plasminogen to plasmin

Most strains of group A streptococci produce a number of other extracellular products including streptokinase, hyaluronidase, nucleases, and a **C5a peptidase**. The C5a peptidase is an enzyme that degrades complement component C5a, the main factor that attracts phagocytes to sites of complement deposition. The enzymatic actions of the others likely play some role in tissue injury or spread, but no specific roles have been defined. Some are antigenic and have been the basis of serologic tests. **Streptokinase** causes lysis of fibrin clots through conversion of plasminogen in normal plasma to the protease plasmin.

## GROUP A STREPTOCOCCAL DISEASE

CLINICAL CAPSULE

Group A streptococci are the cause of "strep throat," an acute inflammation of the pharynx and tonsils that includes fever and painful swallowing. Skin and soft tissue infections range from the tiny skin pustules called impetigo to a severe toxic and invasive disease that can be fatal in a matter of days. In addition to acute infections, group A streptococci are responsible for inflammatory diseases that are not direct infections but represent states in which the immune response to streptococcal antigens causes injury to host tissues. Acute rheumatic fever (ARF) is a prolonged febrile inflammation of connective tissues, which recurs following each subsequent streptococcal pharyngitis. Repeated episodes cause permanent scarring of the heart valves. Acute glomerulonephritis is an insidious disease with hypertension, hematuria, proteinuria, and edema due to inflammation of the renal glomerulus.

## EPIDEMIOLOGY

### Pharyngitis

Most common bacterial cause of sore throat

Droplets spread over short distances from throat and nasal sites

Group A streptococci are the most common bacterial cause of pharyngitis in school-age children 5 to 15 years of age. Transmission is person to person from the large droplets produced by infected persons during coughing, sneezing, or even conversation. This droplet transmission is most efficient at the short distances (2 to 5 feet) at which social interactions commonly take place in families and schools, particularly in fall and winter months. Asymptomatic carriers ($<$1%) may also be the source particularly if colonized in the nose as well as the throat. Although group A streptococci survive for some time in dried secretions, environmental sources and fomites are not important means of spread. Unless the condition is treated, the organisms persist for 1 to 4 weeks after symptoms have disappeared.

## Impetigo

Impetigo occurs when transient skin colonization with group A streptococci is combined with minor trauma such as insect bites. The tiny skin pustules are spread locally by scratching and to others by direct contact or shared fomites such as towels. Impetigo is most common in summer months when insects are biting and when the general level of hygiene is low. The M protein types of *S. pyogenes* most commonly associated with impetigo are different from those causing respiratory infection.

Skin colonization plus trauma leads to impetigo

## Wound and Puerperal Infections

Group A streptococci, once a leading cause of postoperative wound and puerperal infections, retain this potential, but these conditions are now less common. As with staphylococci, transmission from patient to patient is by the hands of physicians or other medical attendants who fail to follow recommended handwashing practices. Organisms may be transferred from another patient or come from the health care workers themselves.

Hospital outbreaks are linked to carriers

## Streptococcal Toxic Shock Syndrome

Since the late 1980s, a severe invasive form of group A streptococcal soft tissue infection appeared with increased frequency (5 to 10 cases/100,000) in the United States and other countries. Rapid progression to death in only a few days occurred in previously healthy persons, including Muppet creator Jim Henson (of Sesame Street fame). The outstanding features of these infections are their multiorgan involvement suggesting a toxin and rapid invasiveness with spread to the bloodstream and distant organs. The toxic features together with the discovery that almost all the isolates produce one of the SPEs have caused this syndrome to be labeled streptococcal toxic shock syndrome (STSS).

STSS may be fatal in healthy persons

Strains produce SPEs

## Poststreptococcal Sequelae

The association between group A streptococci and the inflammatory disease acute rheumatic fever (ARF; see the text) is based on epidemiologic studies linking group A streptococcal pharyngitis, the clinical features of rheumatic fever, and heightened immune responses to streptococcal products. ARF does not follow skin or other nonrespiratory infection with group A streptococci. Although some M types may be more "rheumatogenic," it is generally believed that recurrences of ARF can be triggered by infection with any group A streptococcus. Injury to the heart caused by recurrences of ARF leads to **rheumatic heart disease,** a major cause of heart disease worldwide. Although ARF has declined in developed countries ($<$0.5 cases/1000), a resurgence in the form of small regional outbreaks in the United States began in the late 1980s. These outbreaks involved children of a higher socioeconomic status than previously associated with ARF and a shift in prevalent M types. The underlying basis of the resurgence is unknown.

ARF follows respiratory, not skin, infection

Rheumatic heart disease is produced by recurrent ARF

Poststreptococcal glomerulonephritis may follow either respiratory or cutaneous group A streptococcal infection and involves only certain "nephritogenic" strains. It is more common in temperate climates where insect bites lead to impetigo. The average latent period between infection and glomerulonephritis is 10 days from a respiratory infection, but generally about 3 weeks from a skin infection. Nephritogenic strains are limited to a few M types and seem to have declined in recent years.

Glomerulonephritis follows respiratory or skin infection

Only nephritogenic strains are involved

# PATHOGENESIS

## Acute Infections

As with other pathogens, adherence to mucosal surfaces is a crucial step in initiating disease. A dozen adhesins have been described that facilitate the ability of the group A streptococcus to adhere to epithelial cells of the nasopharynx and/or skin. Of these, the most important are M protein, LTA, and protein F. In the nasopharynx, all three appear to be involved in mediating attachment to the fatty acid–binding sites in the

Surface molecules binding to fibronectin is important first step

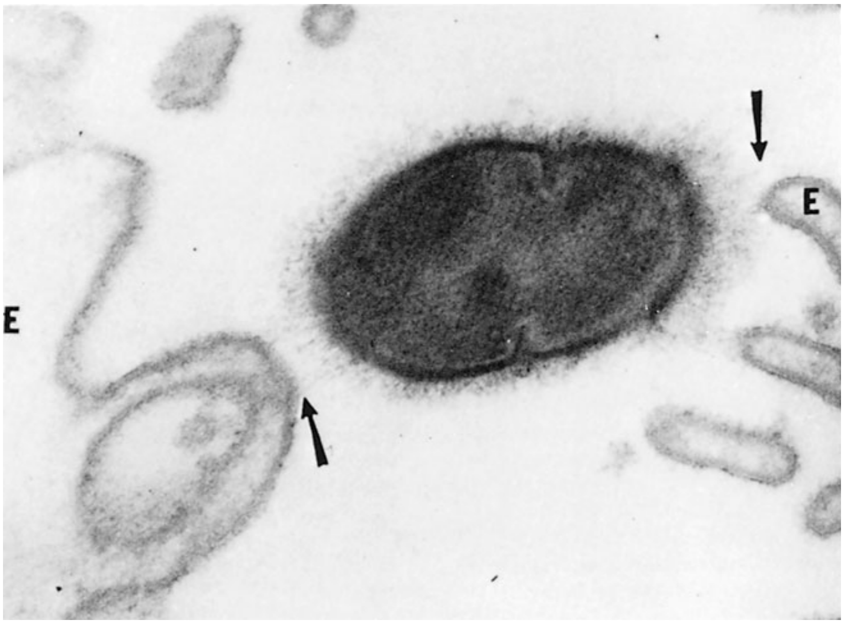


FIGURE 17–3
A group A $\beta$-hemolytic streptococcus is shown attaching to the cell membrane of a human oral epithelial cell (E). Note the hairlike pili (*arrows*), which mediate the attachment. As in Figure 16–1, both M protein and lipoteichoic acid are associated with the pili. (*Reproduced with permission from Beachey EH, Ofek I.* J Exp Med *1976;143:764. Figure 2.*)

M protein supports nasopharyngeal cell adherence

glycoprotein fibronectin covering the epithelial cell surface. The role of M protein is not direct but it appears to provide a scaffold for LTA, which is essential for it to reach its binding site (Fig 17–3).

M protein and protein F are involved in epidermis binding

Expression is environmentally regulated by $O_2$ and $CO_2$

On the other hand, M protein appears to be direct and dominant in binding to the skin through its ability to interact with subcorneal keratinocytes, the most numerous cell type in cutaneous tissue. This adherence takes place at domains of the M protein that bind to CD46 and possibly other receptors on the keratinocyte surface. Protein F is also involved primarily in adherence to antigen-presenting Langerhans cells. Expression of M protein and protein F is environmentally regulated in response to changing concentrations of $O_2$ and $CO_2$. Experimental evidence suggests that a high $O_2$ environment favors protein F and adherence to Langerhans cells, while an environment richer in $CO_2$ favors M protein synthesis and interaction with keratinocytes. This environmentally controlled sequential interaction of *S. pyogenes* with different types of host cells should play some mitigating role either in establishing the microbe or in altering the development of a normally protective host response.

Multiple factors are involved in invasion

Clinical evidence makes it clear that group A streptococci have the capacity to be highly invasive. The events following attachment that trigger invasion are only starting to be understood. It appears that M protein, protein F, and other fibronectin-binding proteins are required for invasion of nonprofessional phagocytes. This invasion involves integrin receptors and is accompanied by cytoskeleton rearrangements but the molecular events do not yet make a coherent story.

Antiphagocytic M protein binds fibrinogen and factor H

Surface C3b deposition is diminished

C5a peptidase blocks phagocyte chemotaxis

After the initial events of attachment and invasion, it appears that the concerted activity of the M protein, immunoglobulin-binding proteins, and the C5a peptidase play the key roles in allowing the streptococcal infection to continue. M protein plays an essential role in group A streptococcal resistance to phagocytosis. The antiphagocytic activity of M protein is related to the ability of domains of the molecule to bind fibrinogen and serum factor H. This leads to a diminished availability of alternative pathway generated complement component C3b for deposition on the streptococcal surface (Fig 17–4). In the presence of M type–specific antibody, classical pathway opsonophagocytosis proceeds, and the streptococci are rapidly killed. As a second antiphagocytic mechanism the C5a peptidase inactivates C5a and thus blocks chemotaxis of polymorphonuclear neutrophils (PMNs) and other phagocytes to the site of infection. Although the hyaluronic acid capsule contributes to resistance to phagocytosis, the mechanisms involved are unknown.

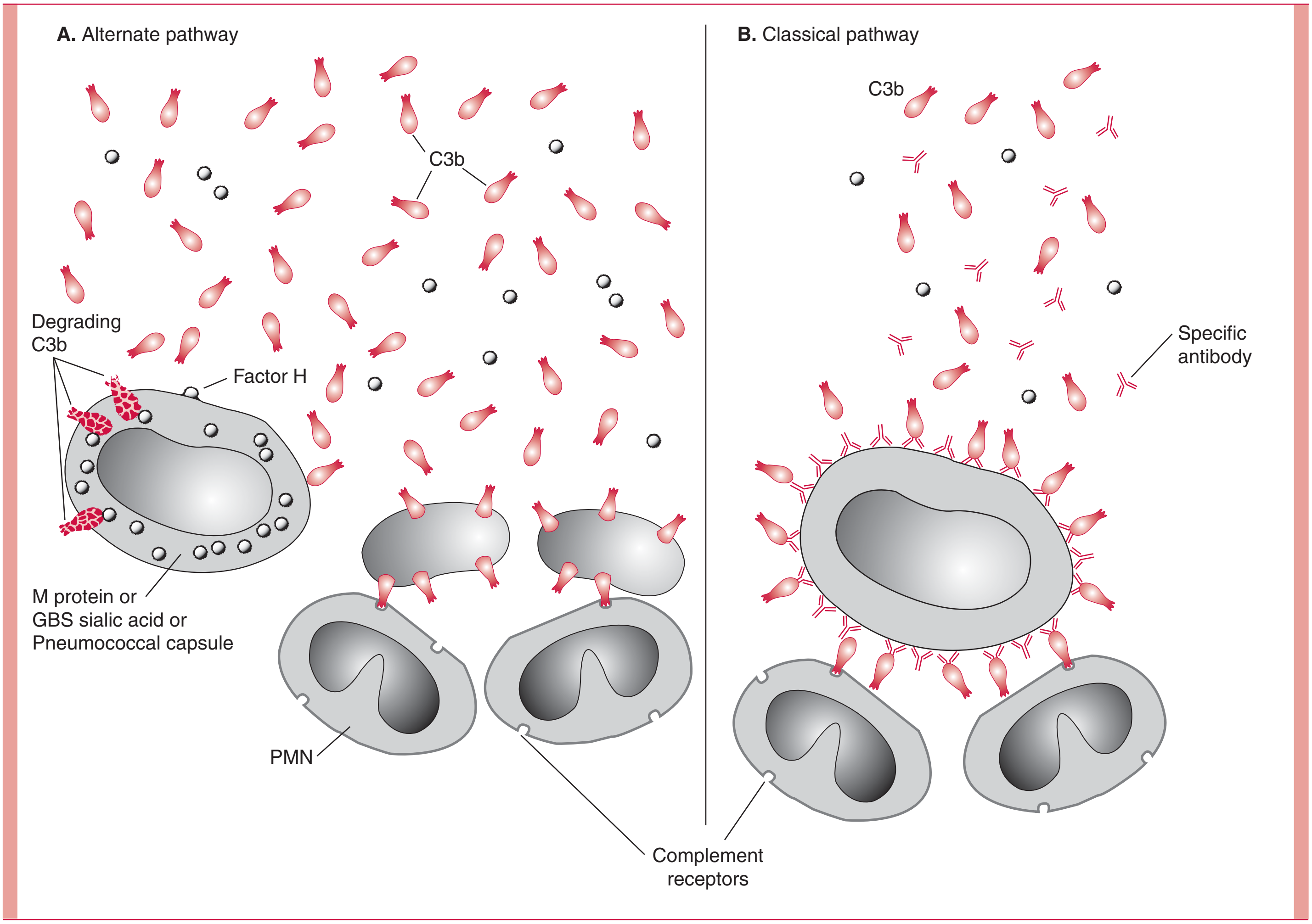


FIGURE 17–4

Streptococcal resistance to opsonophagocytosis. **A.** Alternate pathway. In the alternate complement pathway, C3b binds to the surface of bacteria, providing a recognition site for professional phagocytes and sometimes causing direct injury. Streptococci with special surface structures such as capsules or M protein are able to bind serum factor to their surface. This interferes with complement deposition by accelerating the breakdown of C3b. **B.** Classical pathway. Specific antibody binding to an antigen on the surface provides another binding cite for C3b. Phagocyte recognition may occur even if factor H is present.

The precise role of other bacterial factors in the pathogenesis of acute infection is uncertain, but the combined effect of streptokinase, DNAase, and hyaluronidase may prevent effective localization of the infection, while the streptolysins produce tissue injury and are toxic to phagocytic cells. If any of the SPEs are produced, they may contribute as well but their presence is not essential for acute infections. Antibodies against these components are formed in the course of streptococcal infection but are not known to be protective.

Other virulence factors contribute to spread and injury

In **streptococcal toxic shock syndrome (STSS),** as with staphylococcal toxic shock syndrome, the findings of shock, renal impairment, and diarrhea seem to be explained by the massive cytokine release stimulated by the superantigenicity of the SPEs. Exotoxin production, however, does not easily explain the enhanced invasiveness of group A streptococci, which is an added feature of STSS compared to its staphylococcal counterpart. The enzymatic activity of SPE-B has been linked to invasiveness, but strains with SPE-A and SPE-C are much more common in STSS than SPE-B. This syndrome may represent bacteriophage-mediated horizontal transfer of the SPE genes among recently emerged clones with enhanced invasive potential, a deadly combination. The basis of the enhanced invasiveness remains to be determined.

Superantigenicity of SPEs contributes to STSS

Invasive component is unexplained

## Poststreptococcal Sequelae

### *Acute Rheumatic Fever (ARF)*

ARF is an autoimmune state induced by streptococcal infection

Of the many theories advanced to explain the role of group A streptococci in ARF, an autoimmune mechanism related to antigenic similarities between streptococci and human tissue antigens has the most experimental support. Streptococcal pharyngitis patients who develop ARF have higher levels of antistreptococcal and autoreactive antibodies and T cells than those who do not. Some of these have been shown to react with both heart tissue and streptococcal antigens.

Antistreptococcal antibodies cross-react with heart sarcolemma

M protein epitopes differ from antiphagocytic domains

Antibodies to group A carbohydrate react with valves

The antigen stimulating these antibodies is most probably M protein, but the group A carbohydrate is also a possibility. The similarity between the structure of M protein and myosin is an obvious connection, and M protein fragments have been shown to stimulate antibodies that bind to human heart sarcolemma membranes. Immunochemical studies of M proteins from different M types are now directed at defining unique epitopes responsible for ARF and the extent to which they are shared between serotypes and strains. Domains of the M protein molecule responsible for the heart cross-reactivity have been identified, which differ from those responsible for the factor H and fibrinogen binding. Thus, the cross-reactive and antiphagocytic properties of M protein appear to reside in separate parts of the molecule (see Fig 17–2). Antibodies to the dominant epitope of the group A carbohydrate (N-acetylglucosamine) may play a role in injury to the valvular endothelium, but T cells stimulated by M protein have been seen in valves as well.

Cell-mediated immunity responses include cytotoxic lymphocytes

ARF patients also show enhanced cell-mediated immune responses to streptococcal antigens. Cytotoxic T lymphocytes may be stimulated by M protein, and cytotoxic lymphocytes have been observed in the blood of patients with ARF. A cellular reaction pattern consisting of lymphocytes and macrophages aggregated around fibrinoid deposits is found in human hearts. This lesion, called the **Aschoff body,** is considered characteristic of rheumatic carditis. Suggestions that M protein has superantigen properties must still be reconciled with the prolonged nature of the illness.

Alloantigens are associated with hyperreactivity to streptococci

Genetic factors are probably also important in ARF because only a small proportion of individuals infected with group A streptococci develop the disease. Attack rates have been highest among those of lower socioeconomic status and vary among those of different racial origins. The gene for an alloantigen found on the surface of B lymphocytes occurs among rheumatic fever patients at a frequency fourfold to fivefold greater than the general population. This further suggests a genetic predisposition to hyperreactivity to streptococcal products.

### *Acute Glomerulonephritis*

Autoimmune reactions to M protein or streptokinase are implicated

The renal injury of acute glomerulonephritis is caused by deposition in the glomerulus of antigen–antibody complexes with complement activation and consequent inflammation. The M proteins of some nephritogenic strains have been shown to share antigenic determinants with glomeruli, which suggests an autoimmune mechanism similar to rheumatic fever. Streptokinase has also been implicated both through molecular mimicry and through its plasminogen activation capacity.

## IMMUNITY

Type-specific IgG reverses antiphagocytic effect of M protein

Repeated infections and ARF are due to many M types

It has long been known that antibody directed against M protein is protective for subsequent group A streptococcal infections. This protection, however, is only for subsequent infection with strains of the same M type. This is called **type-specific immunity.** This protective IgG is directed against epitopes in the amino-terminal regions of the molecule and reverses the antiphagocytic effect of M protein. Streptococci opsonized with type-specific antibody bind complement C3b by the classical mechanism, facilitating phagocyte recognition (see Fig 17–4). There is evidence that mucosal IgA is also important in blocking adherence while the IgG is able to protect against invasion. Unfortunately, because there are over 80 M types, repeated infections with other M types occurs. Eventually, immunity to the common M types is acquired and infections become less common in adults. In ARF patients, it is the hyperreaction seen in each episode that produces the lesions associated with rheumatic heart disease.

# GROUP A STREPTOCOCCAL INFECTIONS: CLINICAL ASPECTS

## MANIFESTATIONS

### Streptococcal Pharyngitis

Although it may occur at any age, streptococcal pharyngitis is most frequent between the ages of 5 and 15 years. The illness is characterized by acute sore throat, malaise, fever, and headache. Infection typically involves the tonsillar pillars, uvula, and soft palate, which become red, swollen, and covered with a yellow-white exudate. The cervical lymph nodes that drain this area may also become swollen and tender. Group A streptococcal pharyngitis is usually self-limiting. Typically, the fever is gone by the third to fifth day, and other manifestations subside within 1 week. Occasionally the infection may spread locally to produce peritonsillar or retropharyngeal abscesses, otitis media, suppurative cervical adenitis, and acute sinusitis. Rarely, more extensive spread occurs, producing meningitis, pneumonia, or bacteremia with metastatic infection in distant organs. In the preantibiotic era, these suppurative complications were responsible for a mortality of 1 to 3% following acute streptococcal pharyngitis. Such complications are much less common now, and fatal infections are rare.

Strep throat syndrome overlaps with viral pharyngitis

Spread beyond the pharynx uncommon

### Impetigo

The primary lesion of streptococcal impetigo is a small (up to 1 cm) vesicle surrounded by an area of erythema. The vesicle enlarges over a period of days, becomes pustular, and eventually breaks to form a yellow crust. The lesions usually appear in 2- to 5-year-old children on exposed body surfaces, typically the face and lower extremities. Multiple lesions may coalesce to form deeper ulcerated areas. Although *S. aureus* produces a clinically distinct bullous form of impetigo (see Chapter 16), it can also cause vesicular lesions resembling streptococcal impetigo. Both pathogens are isolated from some cases.

Exposed skin of 2- to 5-year-old children

Tiny pustules may combine to form ulcers

### Erysipelas

Erysipelas is a distinct form of streptococcal infection of the skin and subcutaneous tissues, primarily affecting the dermis. It is characterized by a spreading area of erythema and edema with rapidly advancing, well-demarcated edges, pain, and systemic manifestations, including fever and lymphadenopathy. Infection usually occurs on the face (Fig 17–5), and a previous history of streptococcal sore throat is common.

Spreading erythema of dermal tissues

### Puerperal Infection

Infection of the endometrium at or near delivery is a life-threatening form of group A streptococcal infection. Fortunately, it is now relatively rare, but in the 19th century, the clinical findings of "childbed fever" were characteristic and common enough to provide the first clues to the transmission of bacterial infections in hospitals (see Chapter 72). Other organisms can cause puerperal fever, but this form is the most likely to produce a rapidly progressive infection.

Group A streptococcus causes the most virulent form of puerperal fever

### Disease Associated with Streptococcal Pyrogenic Exotoxins

#### *Scarlet Fever*

Infection with strains that elaborate any of the SPEs may superimpose the signs of scarlet fever on a patient with streptococcal pharyngitis. In scarlet fever, the buccal mucosa, temples, and cheeks are deep red, except for a pale area around the mouth and nose (circumoral pallor). Punctate hemorrhages appear on the hard and soft palates, and the tongue becomes covered with a yellow-white exudate through which the red papillae are prominent (strawberry tongue). A diffuse red "sandpaper" rash appears on the second day

Scarlet fever is strep throat with a characteristic rash

FIGURE 17–5
Streptococcal erysipelas. The diffuse erythema and swelling in the face of this woman are characteristic of group A streptococcal cellulitis at any site. (*Reproduced with permission from Connor DH, Chandler FW, Schwartz DQA, Manz HJ, Lack EE (eds).* Pathology of Infectious Diseases, *vol. 1. Stamford, CT: Appleton & Lange; 1997.*)

of illness, spreading from the upper chest to the trunk and extremities. Circulating antibody to the toxin neutralizes these effects. For unknown reasons, scarlet fever is both less frequent and less severe than early in the 20th century.

### *Streptococcal Toxic Shock Syndrome (STSS)*

STSS is a rapidly progressive multisystem disease

Shock, azotemia, and bacteremia are common

STSS may begin at the site of any group A streptococcal infection even at the site of seemingly minor trauma. The systemic illness starts with vague myalgia, chills, and severe pain at the infected site. Most commonly, this is in the skin and soft tissues and leads to necrotizing fasciitis and myonecrosis. The striking nature of this progression when it involves the extremities is the basis of the label "flesh-eating bacteria." STSS continues with nausea, vomiting, and diarrhea followed by hypotension, shock, and organ failure. The outstanding laboratory findings are a lymphocytosis; impaired renal function (azotemia); and, in over half the cases, bacteremia. Some patients are in irreversible shock by the time they reach a medical facility. Many survivors have been left as multiple amputees as the result of metastatic spread of the streptococci.

## Poststreptococcal Sequelae

### *Acute Rheumatic Fever (ARF)*

ARF causes inflammation of connective tissue and endocardium

Infection with new M types triggers recurrences

Recurrences lead to heart valve damage

ARF is a nonsuppurative inflammatory disease characterized by fever, carditis, subcutaneous nodules, chorea, and migratory polyarthritis. Cardiac enlargement, valvular murmurs, and effusions are seen clinically and reflect endocardial, myocardial, and epicardial damage, which can lead to heart failure. Attacks typically begin 3 weeks (range, 1 to 5 weeks) after an attack of group A streptococcal pharyngitis, and in the absence of antiinflammatory therapy last 2 to 3 months. ARF also has a predilection for recurrence with subsequent streptococcal infections as new M types are encountered. The first attack usually occurs between the ages of 5 and 15 years. The risk of recurrent attacks after subsequent group A streptococcal infections continues into adult life and then decreases. Repeated attacks lead to progressive damage to the endocardium and heart valves, with scarring and valvular stenosis or incompetence (rheumatic heart disease).

### Acute Glomerulonephritis

Poststreptococcal glomerulonephritis is primarily a disease of childhood that begins 1 to 4 weeks after streptococcal pharyngitis and 3 to 6 weeks after skin infection. It is characterized clinically by edema, hypertension, hematuria, proteinuria, and decreased serum complement levels. Pathologically, there are diffuse proliferative lesions of the glomeruli. The clinical course is usually benign, with spontaneous healing over weeks to months. Occasionally, a progressive course leads to renal failure and death.

Children develop a nephritis, which slowly resolves

## DIAGNOSIS

Although these clinical features of streptococcal pharyngitis are fairly typical, there is enough overlap with viral pharyngitis that a culture of the posterior pharynx and tonsils is required for diagnosis. A direct Gram-stained smear of the throat is unhelpful because of the other streptococci in the pharyngeal flora, but smears from normally sterile sites usually demonstrate streptococci. Blood agar plates incubated anaerobically give the best yield because they favor the demonstration of $\beta$-hemolysis (see streptolysins, above). $\beta$-hemolytic colonies are identified by Lancefield grouping using immunofluorescence or agglutination methods. In smaller laboratories, an indirect method based on the exquisite susceptibility of group A strains to bacitracin and the relative resistance of strains of other groups may be used for presumptive separation of group A strains from the others (Table 17–2).

Throat culture followed by Lancefield grouping is definitive

Bacitracin susceptibility predicts group A

Detection of group A antigen extracted directly from throat swabs is now available in a wide variety of kits marketed for use in physicians' offices. These methods are rapid and specific, but most are only 90 to 95% sensitive compared to culture. Given the importance of the detection of group A streptococci in prevention of ARF (the reason physicians culture sore throats), missing even 5% of cases is not tolerable. Until direct antigen detection methods gain a higher sensitivity, negative results must be confirmed by culture before withholding treatment. Some of the newer antigen detection procedures are approaching a sensitivity that would allow their substitution for culture.

Direct detection of A antigen is rapid

Several serologic tests have been developed to aid in the diagnosis of poststreptococcal sequelae by providing evidence of a previous group A streptococcal infection. They

TABLE 17–2

**Usual Hemolytic, Biochemical, and Cultural Reactions of Common Streptococci and Enterococci[a]**

| | Susceptibility to | | Bile | Bile/Esculin | |
|---|---|---|---|---|---|
| | Bacitracin | Optochin | Solubility | Reaction[b] | PYR[c] |
| **Streptococci** | | | | | |
| $\beta$-Hemolytic | | | | | |
| Lancefield group A | + | – | – | – | + |
| Lancefield groups B, C, F, G | – | – | – | – | – |
| $\alpha$-Hemolytic | | | | | |
| *S. pneumoniae* | – | + | + | – | – |
| Viridans group | – | – | – | – | – |
| Nonhemolytic | – | – | – | – | – |
| **Enterococci** | – | – | – | + | + |

[a] All are tests commonly substituted for serologic identification in clinical laboratories.

[b] Tests for the ability to grow in bile and reduce esculin.

[c] PYR = pyrrolidonyl arylamidase test.

ASO antibodies document previous infection in suspect ARF

include the ASO, anti-DNAase B, and some tests that combine multiple antigens. High titers of ASO are usually found in sera of patients with rheumatic fever, so that test is used most widely.

## TREATMENT

Group A streptococci remain susceptible to penicillin

Group A streptococci are highly susceptible to penicillin G, the antimicrobic of choice. Concentrations as low as 0.01 $\mu$g/mL have a bactericidal effect, and penicillin resistance is so far unknown. Numerous other antimicrobics are also active, including other penicillins, cephalosporins, tetracyclines, and macrolides, but not aminoglycosides.

Treatment of pharyngitis within 10 days prevents ARF

Patients allergic to penicillin are usually treated with erythromycin if the organisms are susceptible. Impetigo is often treated with erythromycin to cover the prospect of *S. aureus* involvement. Adequate treatment of streptococcal pharyngitis within 10 days of onset prevents rheumatic fever by removing the antigenic stimulus; its effect on the duration of the pharyngitis is less, because of the short course of the natural infection. Treatment does not prevent the development of acute glomerulonephritis.

## PREVENTION

Prophylactic penicillin prevents ARF recurrences

Penicillin prophylaxis with long-acting preparations is used to prevent recurrences of ARF during the most susceptible ages (5 to 15 years). Patients with a history of rheumatic fever or known rheumatic heart disease receive antimicrobial prophylaxis while undergoing procedures known to cause transient bacteremia, such as dental extraction. Vaccines using epitopes of the M protein molecule, which would provide protection against acute infection without stimulating autoantibodies are in development. This is a sizable task given the large number of M protein serotypes.

# *Group B Streptococci* (Streptococcus agalactiae)

## BACTERIOLOGY

Nine capsular types contain sialic acid

Group B streptococci (GBS) produce short chains and diplococcal pairs of spherical or ovoid Gram-positive cells. Colonies are larger and $\beta$-hemolysis is less distinct than with group A streptococci and may even be absent. In addition to the Lancefield B antigen, GBS produce polysaccharide capsules of nine antigenic types (Ia, Ib, II through VIII) all of which contain sialic acid in the form of terminal side chain residues.

## GROUP B STREPTOCOCCAL DISEASE

CLINICAL CAPSULE

The typical GBS case is a newborn in the first few days of life who is not doing well. Fever, lethargy, poor feeding, and respiratory distress are the most common features. Localizing findings are usually lacking, and the diagnosis is revealed only by isolation of GBS from blood or cerebrospinal fluid. The mortality rate is high even when appropriate antibiotics are used.

## EPIDEMIOLOGY

Neonatal sepsis is acquired from mother's vaginal flora

GBS are the leading cause of sepsis and meningitis in the first few days of life. The organism is resident in the gastrointestinal tract, with secondary spread to other sites, the most important of which is the vagina. GBS can be found in the vaginal flora of 10 to 30% of women, and during pregnancy and delivery, these organisms may again access to the amniotic fluid or colonize the newborn as it passes through the birth canal. Judging from US surveillance data (1.8 cases/1000 live births), GBS produce disease in approximately 2% of these encounters. The risk is much higher when factors are present that decrease the infant's innate resistance (prematurity) or increase the chances of transmission (ruptured amniotic membranes). Some infants are healthy at birth but develop sepsis 1 to 3 months later. It is not known whether the organism in these "late-onset" cases was acquired from the mother, in the nursery, or in the community after leaving the hospital.

Ruptured membranes and prematurity increase risk

## PATHOGENESIS

GBS disease requires the proper combination of organism and host factors. The GBS capsule is the major organism factor. The sialic acid moiety of the capsule has been shown to bind serum factor H, which in turn accelerates degradation of C3b before it can be effectively deposited on the surface of the organism. This makes alternate pathway–mediated mechanisms of opsonophagocytosis relatively ineffective (see Fig 17–4). Thus, complement-mediated phagocyte recognition requires specific antibody and the classical pathway. Newborns will have this antibody only if they receive it from their mother as transplacental IgG. Those who lack the protective "cover" of antibody specific to the type of GBS they encounter must rely on alternate pathway mechanisms, a situation in which the GBS has an advantage over less virulent organisms. GBS have also been shown to produce a peptidase that inactivates C5a, the major chemoattractant of PMNs. This may correlate with the observation that serious neonatal infections often show a paucity of infiltrating PMNs.

Capsule binds factor H

C3b deposition is disrupted

Transplacental IgG is protective

## IMMUNITY

Antibody is protective against GBS disease, but as with group A streptococcal M protein, the antibody must be specific to the infecting type of GBS. Fortunately, there are only nine types and type III produces the majority of cases in the first week of life. Antibody is acquired by GBS infection, and specific IgG may be transmitted transplacentally to the fetus, providing protection in the perinatal period. In the presence of type-specific antibody, classical pathway C3b deposition, phagocyte recognition, and killing proceed normally.

Type-specific anticapsular antibody is protective

# GROUP B STREPTOCOCCI: CLINICAL ASPECTS

## MANIFESTATIONS

The clinical findings are nonspecific and similar to those found in other serious infections in the neonatal period (see Chapter 69). Respiratory distress, fever, lethargy, irritability, apnea, and hypotension are common. Fever is sometimes absent, and infants may even be hypothermic. Pneumonia is common, and meningitis is present in 5 to 10% of cases, but most infections have GBS circulating in the bloodstream without localizing findings. The onset is typically in the first few days of life, and signs of infection are present at birth in almost 50% of cases. The late-onset (1 to 3 month) cases have similar findings but are more likely to have meningitis and focal infections in the bones and joints. Even with appropriate and prompt treatment, the mortality rate for early onset GBS infection approaches 20%.

Nonspecific findings evolve to pneumonia and meningitis

First few days of life or months later

GBS infections in adults are uncommon and fall in two groups. The first are peripartum chorioamnionitis and bacteremia, the mother's side of the neonatal syndrome. Other infections include pneumonia and a variety of skin and soft tissue infections similar to those produced by other pyogenic streptococci. Although adult GBS infections may be serious, they are usually not fatal unless patients are immunocompromised. GBS are not associated with rheumatic fever or acute glomerulonephritis.

Maternal and other adult infections can be serious

## DIAGNOSIS

Culture is only standard method

The laboratory diagnosis of GBS infection is by culture of blood, cerebrospinal fluid, or other appropriate specimen. Definitive identification involves serologic determination of the Lancefield group by the same methods used for group A streptococci. Methods for direct detection of GBS antigen in vaginal specimens have been evaluated, but their sensitivity is far too low for use in the diagnosis of neonatal infection.

## TREATMENT

Combinations of $\beta$-lactam and aminoglycoside are used

GBS are susceptible to the same antimicrobics as group A organisms. Although penicillin is the treatment of choice, GBS are slightly less susceptible to $\beta$-lactams than other streptococci. For this reason neonatal infections are often initially treated with combinations of penicillin (or ampicillin) and an aminoglycoside. These combinations have been shown to accelerate killing of GBS in vitro.

## PREVENTION

Intrapartum prophylaxis is protective

Third trimester vaginal culture and/or clinical factors determine risk

Vaccine is a prospect

Current strategies for prevention of neonatal GBS disease are focused on reducing contact of the infant with the organism. In colonized women, attempts to eradicate the carrier state have not been successful, but intrapartum antimicrobial prophylaxis with penicillin or ampicillin has been shown to reduce transmission and disease in high-risk populations. It is now recommended by expert obstetric and perinatology groups that all newborns at risk receive such prophylaxis, but there is debate about the practical aspects of determining risk. One approach is to screen all expectant mothers for vaginal GBS colonization in the third trimester and administer prophylaxis during labor to all found to be culture positive. This safe but expensive approach can be applied only to those who seek regular prenatal care. A second approach is to assign risk on clinical grounds (eg, prematurity, prolonged membrane rupture, fever), which is less expensive but will miss some colonized babies. There is evidence that prophylaxis is working. The incidence of early-onset neonatal GBS disease dropped 65% over a 5-year period when these strategies were being implemented. Prevention by immunization with purified GBS capsular polysaccharide has been shown to be feasible, and considerable effort is now being directed at development of a vaccine.

# *Other Pyogenic Streptococci*

All are virulent but uncommon

None associated with immunologic sequelae

The other pyogenic streptococci occasionally produce various respiratory, skin, wound, soft tissue, and genital infections, which may resemble those caused by group A and B streptococci. Although a few food-borne outbreaks of pharyngitis have been linked to non–group A streptococci, their role as a cause of everyday sore throats is not established. These streptococci are susceptible to penicillin, and infections are managed in a manner similar to deep tissue infections caused by group A and B strains. None of the non–group A streptococci have been associated with poststreptococcal sequelae.

# *Streptococcus pneumoniae*

## BACTERIOLOGY

## MORPHOLOGY AND STRUCTURE

Capsule has 90+ serotypes

*S. pneumoniae* (pneumococci) are Gram-positive, oval cocci typically arranged end to end in pairs (diplococcus) giving the cells a bullet shape (Fig 17–6). The distinguishing structural feature of the pneumococcus is its capsule. All virulent strains have surface capsules,

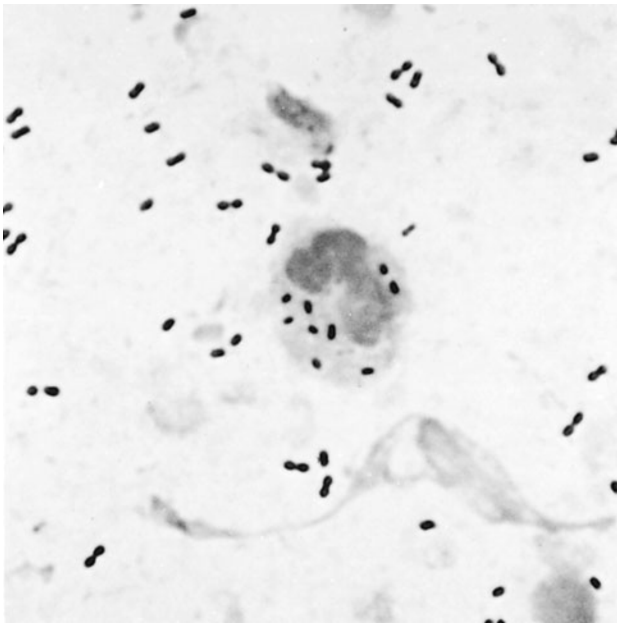

FIGURE 17–6
*Streptococcus pneumoniae* in sputum of patient with pneumonia. Note the marked tendency to form oval diplococci.

composed of high-molecular-weight polysaccharide polymers that are complex mixtures of monosaccharides, oligosaccharides, and sometimes other components. The exact makeup of the polymer is unique and distinctly antigenic for each of more than 90 serotypes. A number of pneumococcal surface proteins have been identified but their function is not known.

Pneumococcal cell wall structure is similar to other streptococci. Teichoic acid, LPA, and phosphocholine are rooted in the peptidoglycan extending outward into the capsule where they provide binding domains for a variety of surface proteins. At least one of these, a **choline binding protein,** is able to bind to both pneumococcal cell wall cholines and carbohydrates present on the surface of epithelial cells.

Choline binding protein attaches to cells

## GROWTH

On blood agar, pneumococci produce round, glistening 0.5- to 2.0-mm colonies surrounded by a zone of $\alpha$-hemolysis. Both colonies and broth cultures have a tendency to undergo autolysis due to their susceptibility to peroxides produced during growth and the action of **autolysins,** a family of pneumococcal enzymes that degrade peptidoglycan. Accelerating the autolytic process with bile salts is the basis of the bile solubility test that separates pneumococci from other $\alpha$-hemolytic streptococci.

Colonies are $\alpha$-hemolytic

## EXTRACELLULAR PRODUCTS

All pneumococci produce **pneumolysin,** which is a member of the family of transmembrane pore-forming toxins that includes staphylococcal $\alpha$ toxin, *S. pyogenes* streptolysin O, and others. The pneumococcus does not secrete pneumolysin but it is released on lysis of the organisms augmented by autolysins. Pneumolysin has a number of other effects, including its ability to stimulate cytokines and disrupt the cilia of cultured human respiratory epithelial cells. Pneumococci also produce a **neuraminidase,** which cleaves sialic acid present in host mucin, glycolipids and glycoproteins.

Pneumolysin forms pores after release by autolysins

# PNEUMOCOCCAL DISEASE

The most common form of infection with *S. pneumoniae* is pneumonia, which begins with fever and a shaking chill followed by signs that localize the disease to the lung. These include difficulty breathing and cough with production of purulent

CLINICAL CAPSULE

sputum, sometimes containing blood. The pneumonia typically fills part or all of a lobe of the lung with inflammatory cells, and the bacteria may spread to the bloodstream and thus other organs. The most important of the latter is the central nervous system, where seeding with pneumococci leads to acute purulent meningitis.

## EPIDEMIOLOGY

Pneumonia is common

Young and old are most affected

*S. pneumoniae* is a leading cause of pneumonia, acute purulent meningitis, bacteremia, and other invasive infections. In the United States it is responsible for an estimated 3000 cases of meningitis, 50,000 cases of bacteremia, and 500,000 cases of pneumonia each year. Worldwide, more than 5 million children die every year from pneumococcal disease. *S. pneumoniae* is also the most frequent cause of otitis media (see Chapter 61), a virtually universal disease of childhood with millions of cases every year. Pneumococcal infections occur throughout life but are most common in the very young (<2 years) and in the old (>60 years). Alcoholism, diabetes mellitus, chronic renal disease, asplenia, and some malignancies are all associated with more frequent and serious pneumococcal infection.

Respiratory colonization is common

Microaerosols transmit person to person

Infections are derived from colonization of the nasopharynx, where pneumococci can be found in 5 to 40% of healthy persons depending on age, season, and other factors. The highest rates are among children in the winter. Respiratory secretions containing pneumococci may be transmitted from person to person by direct contact or from the microaerosols created by coughing and sneezing in close quarters. Such conditions are favored by crowded living conditions, particularly when colonized persons are mixed with susceptible ones, as in child care centers, recruitment barracks, and prisons. As with other bacterial pneumonias, viral respiratory infection and underlying chronic disease are important predisposing factors.

Some serotypes are more common

About 23 of the 90 pneumococcal serotypes produce disease more often than the others. There is also a variation in the age and geographic distribution of cases. These differences are presumably due to enhanced virulence factors in these types, but the specific reasons are not known. These features do not influence the medical management of individual cases but are important in devising prevention strategies such as immunization (see below).

## PATHOGENESIS

Aspiration of colonizing bacteria starts the disease process

Impaired clearance mechanisms enhance susceptibility

Pneumococcal adherence to nasopharyngeal cells involves multiple factors. The primary relationship is the bridging effect of the choline binding protein's attachment to cell wall cholines and carbohydrates covering or exposed on the surface of host epithelial cells. This binding may be aided by the exposure of additional receptors by neuraminidase digestion or pneumolysin stimulated cytokine activation of host cells. Aspiration of respiratory secretions containing these pneumococci is the initial event leading to pneumonia. This must be a common event. Normally, aspirated organisms are cleared rapidly by the defense mechanisms of the lower respiratory tract, including the cough and epiglottic reflexes; the mucociliary "blanket;" and phagocytosis by alveolar macrophages. Host factors that impair the combined efficiency of these defenses can allow pneumococci to reach the alveoli and multiply there. These include chronic pulmonary diseases; damage to bronchial epithelium from smoking or air pollution; and respiratory dysfunction from alcoholic intoxication, narcotics, anesthesia, and trauma.

Capsule interferes with phagocytosis

Pneumolysin causes injury

When organisms reach the alveolus, the involvement of pneumococcal virulence factors appears to operate in two stages. The first stage is early in infection, when the surface capsule of intact organisms acts to block phagocytosis by complement inhibition. This allows the organisms to multiply and spread despite an acute inflammatory response. The second stage occurs when organisms begin to disintegrate and release a number of factors either synthesized by the pneumococcus or part of its structure, thus causing injury. These include pneumolysin, autolysin, and components of the cell wall.

## Capsule

The polysaccharide capsule of *S. pneumoniae* is the major determinant of virulence. Unencapsulated mutants do not produce disease in humans or laboratory animals. Like the GBS capsule, pneumococcal polysaccharide interferes with effective deposition of complement on the organism's surface and thus phagocyte recognition and engulfment. This property is particularly important in the absence of specific antibody, when alternate pathway is the primary means for C3b mediated opsonization. The exact mechanism for interference with C3b deposition (see Fig 17–4) may differ in detail with that of GBS sialic acid and between the capsular polysaccharide polymers of individual pneumococcal serotypes. The net effect is that the complement fragments recognized by phagocyte receptors are not available on the surface of the organism. When antibody binds to the capsular polysaccharide, C3b generated by the classical pathway binds and opsonophagocytosis proceeds efficiently.

Unencapsulated pneumococci are avirulent

Alternate pathway C3b deposition blocked by capsule

## Pneumolysin

Some of the clinical features seen in the course of pneumococcal infections are not explainable by the capsule alone. These include the dramatic abrupt onset, toxicity, fulminant course, and disseminated intravascular coagulation seen in some cases. Pneumolysin's toxicity for pulmonary endothelial cells and direct effect on cilia contributes to the disruption of the endothelial barrier and facilitates the access of pneumococci to the alveoli and eventually their spread beyond into the bloodstream. Pneumolysin also has direct effects on phagocytes and suppresses host inflammatory and immune functions. Injection of purified pneumolysin into the lung of rats causes all of the salient histologic hallmarks of pneumococcal pneumonia. Because pneumolysin is not actively secreted outside the bacterial cell, the action of the autolysins is required to release it.

Pneumolysin disrupts cells and cilia

Lysis required to release from bacterial cell

## Other Virulence Determinants

Although the search for the host epithelial cell's adhesin has been unrewarding, it seems logical that one or more of the surface proteins attached to cell wall teichoic acid are involved. Pneumococcal surface protein A (PspA) is found in virtually all pneumococci and has been shown to interfere with complement deposition. In addition to its role in attachment, neuraminidase may have a role at other stages of disease. Peptidoglycan and teichoic acid components of the cell wall have been shown to stimulate inflammation and cerebral edema in experimental meningitis and may do so at other stages of infection. Along with pneumolysin, these may be responsible for the heightened acute inflammatory response seen in pneumococcal infection, which of itself may be destructive to the host.

PspA and neuraminidase act on cell surface

Peptidoglycan stimulates inflammation

The combined effects of pneumococcal and host factors produce a pneumonia, which progresses through a series of stages. Initial alveolar multiplication produces a profuse outpouring of serous edema fluid, which is then followed by an influx of polymorphonuclear leukocytes (PMNs) and erythrocytes (red blood cells; RBCs). By the second or third day of illness, the lung segment has increased three- to fourfold in weight through accumulation of this cellular, hemorrhagic fluid typically in a single lobe of the lung. In the consolidated alveoli, neutrophils predominate initially, but once actively growing pneumococci are no longer present, macrophages replace the granulocytes and resolution of the lesion ensues. A remarkable feature of pneumococcal pneumonia is the lack of structural damage to the lung, which usually leads to complete resolution on recovery.

PMNs and RBCs consolidate alveoli

Lesions resolve without structural damage

# IMMUNITY

Immunity to *S. pneumoniae* infection is provided by antibody directed against the specific pneumococcal capsular type. When antibody binds to the capsular surface, C3b is deposited by classical pathway mechanisms, and phagocytosis can proceed. Because the number of serotypes is large, complete immunity through natural experience is not realistic, which is why pneumococcal infections occur throughout life. Infections are most often

Immunity is specific to capsular type

Antibody leads to classical pathway complement deposition

seen in the very young, when immunologic experience is minimal, and in the elderly, when immunity begins to wane and risk factors are more common. Antibodies to surface proteins and enzymes, including pneumolysin, are also formed in the course of disease, but their role in immunity is unknown.

# PNEUMOCOCCAL DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

### Pneumococcal Pneumonia

Shaking chill is followed by bloody sputum

Lung consolidation is typically lobar

Clinically, pneumococcal pneumonia begins abruptly with a shaking chill and high fever. Cough with production of sputum pink to rusty in color (indicating the presence of RBCs) and pleuritic chest pain are common. Physical findings usually indicate pulmonary consolidation. Children and young adults typically demonstrate a lobular or lobar consolidation on chest radiography, whereas older patients may show a less localized bronchial distribution of the infiltrates. Without therapy, sustained fever, pleuritic pain, and productive cough continue until a "crisis" occurs 5 to 10 days after onset of the disease. The crisis involves a sudden decrease in temperature and improvement in the patient's condition. It is associated with effective levels of opsonizing antibody reaching the lesion. Although infection may occur at any age, the incidence and mortality of pneumococcal pneumonia increase sharply after 50 years.

### Pneumococcal Meningitis

Sequelae are slightly higher than other meningeal pathogens

*S. pneumoniae* is one of the three leading causes of bacterial meningitis. The signs and symptoms are similar to those produced by other bacteria (see Chapter 67). Acute purulent meningitis may follow pneumococcal pneumonia, infection at another site, or appear with no apparent antecedent infection. It may also develop after trauma involving the skull. The mortality and frequency of sequelae are slightly higher with pneumococcal meningitis than with other forms of pyogenic meningitis.

### Other Infections

Sinusitis and otitis media are common

Pneumococci are common causes of sinusitis and otitis media (see Chapter 61). The latter frequently occurs in children in association with viral infection. Chronic infection of the mastoid or respiratory sinus sometimes extends to the subarachnoid space to cause meningitis. Pneumococci may also cause endocarditis, arthritis, and peritonitis, usually in association with bacteremia. Patients with ascites caused by diseases such as cirrhosis and nephritis may develop spontaneous pneumococcal peritonitis. Pneumococci do not cause pharyngitis or tonsillitis.

## DIAGNOSIS

Sputum quality complicates diagnosis

Gram smears of material from sputum and other sites of pneumococcal infection typically show Gram-positive, lancet-shaped diplococci (see Fig 17–6). Sputum collection may be difficult, however, and specimens contaminated with respiratory flora are useless for diagnosis. Other types of lower respiratory specimens may be needed for diagnosis (see Chapters 15 and 64).

Optochin or bile solubility distinguish from viridans streptococci

*S. pneumoniae* grows well overnight on blood agar medium and is usually distinguished from viridans streptococci by susceptibility to the synthetic chemical ethylhydrocupreine (Optochin) or by a bile solubility (see Table 17–2). Bacteremia is common in pneumococcal pneumonia and meningitis, and blood cultures are valuable supplements to cultures of local fluids or exudates. Detection of pneumococcal capsular antigen in body fluids is possible but valuable primarily when cultures are negative.

## TREATMENT

For decades pneumococci were uniformly susceptible to penicillin at concentrations below 0.1 $\mu$g/mL. In the late 1960s, this began to change, and strains with decreased susceptibility to all $\beta$-lactams began to emerge. These strains have penicillin minimal inhibitory concentrations (MICs) of 0.12 to 8.0 $\mu$g/mL and are associated with treatment failures in cases of pneumonia and meningitis. The resistance is not absolute and can be overcome with increased dosage depending on the MIC. The mechanism involves alterations in the $\beta$-lactam target, the transpeptidases that cross-link peptidoglycan in cell wall synthesis. Resistant strains have mutations in one or more of these transpeptidases, which cause decreased affinity for penicillin and other $\beta$-lactams. Penicillinase is not produced. Resistance to erythromycin is uncommon but more likely with penicillin-resistant strains.

Altered transpeptidases decrease penicillin susceptibility

Penicillin is still the antimicrobic of choice for susceptible strains but resistance rates now exceed 10% in most locales and may be greater than 30% in some areas. Penicillin-resistant strains may be treated with erythromycin, vancomycin, or quinolones, if susceptible. Despite the $\beta$-lactam cross resistance, high doses of third-generation cephalosporins have also been used in situations such as meningitis, where their added spectrum may be an advantage. The therapeutic response to treatment of pneumococcal pneumonia is often (but not always) dramatic. Reduction in fever, respiratory rate, and cough can occur in 12 to 24 hours but may occur gradually over several days. Chest radiography may yield normal results only after several weeks.

High doses of third-generation cephalosporins may overcome resistance

## PREVENTION

A vaccine prepared from capsular polysaccharide extracted from the 23 most common serotypes of *S. pneumoniae* is available. This vaccine is presently recommended for patients who are particularly susceptible to pneumococcal infection because of advanced age, underlying disease, or immune status. As with other polysaccharide vaccines it is poorly immunogenic in infants. The newest vaccines follow the success of *Haemophilus influenzae* type b (see Chapter 24) by conjugating the polysaccharide to protein in order to stimulate T-cell dependent responses. This task is greatly complicated by the multiple serotype-specific polysaccharides involved in *S. pneumoniae* disease. A seven-valent conjugate vaccine is now available and recommended for use beginning at 2 months of age. The polysaccharide continues to be recommended beyond the age of 5 years.

23-valent polysaccharide vaccine is available

Protein conjugate vaccine is recommended for children

# *Viridans and Nonhemolytic Streptococci*

The viridans group comprises all $\alpha$-hemolytic streptococci that remain after the criteria for defining pyogenic streptococci and pneumococci have been applied. Characteristically members of the normal flora of the oral and nasopharyngeal cavities, they have the basic bacteriologic features of streptococci but lack the specific antigens, toxins, and virulence of the other groups. Although the viridans group includes many species (see Table 17–2), they are usually not completely identified in clinical practice because there is little difference among them in medical significance.

"Left over" $\alpha$-hemolytic species are in respiratory flora

Although their virulence is very low, viridans strains can cause disease when they are protected from host defenses. The prime example is subacute bacterial endocarditis. In this disease, viridans streptococci reach previously damaged heart valves as a result of transient bacteremia associated with manipulations, such as tooth extraction, that disturb their usual habitat. Protected by fibrin and platelets, they multiply on the valve, causing local and systemic disease that is fatal if untreated. Extracellular production of glucans, complex polysaccharide polymers, may enhance their attachment to cardiac valves in a manner similar to the pathogenesis of dental caries by *S. mutans* (see Chapter 62). The clinical course of viridans streptococcal endocarditis is subacute, with slow progression over weeks or months (see Chapter 68). It is effectively treated with penicillin, but uniformly fatal if untreated.

Low virulence species may cause bacterial endocarditis

Glucan production enhances attachment

The disease is particularly associated with valves damaged by recurrent rheumatic fever. The decline in the occurrence of rheumatic heart disease has reduced the incidence of this particular type of endocarditis.

# ENTEROCOCCI

## BACTERIOLOGY

Formerly called streptococci, enterococci possess group D antigen

Intestinal inhabitants resist action of bile salts

Until DNA homology studies dictated their separation into the genus *Enterococcus,* the enterococci were classified as streptococci. Indeed, the most common enterococcal species share the bacteriologic characteristics described above for pyogenic streptococci, including presence of the Lancefield group D antigen. The term enterococcus derives from their presence in the intestinal tract and the many biochemical and cultural features that reflect that habitat. These include the ability to grow in the presence of high concentrations of bile salts and sodium chloride. Most enterococci produce nonhemolytic or $\alpha$-hemolytic colonies that are larger than those of most streptococci. *E. faecalis, E. faecium,* and several other species are recognized based on biochemical and cultural reactions, but enterococci are generally not speciated in the clinical laboratory.

## ENTEROCOCCAL DISEASE

CLINICAL CAPSULE

Enterococci cause infection almost exclusively in hospitalized patients with significant compromise of their defenses. The primary sites are the urinary tract and soft tissue sites adjacent to the intestinal flora where enterococcal species are resident. The infections themselves are often low grade and have no unique clinical features.

### EPIDEMIOLOGY

Endogenous infection is associated with medical procedures

Enterococci are part of the normal intestinal flora. Although they are capable of producing disease in many settings, the hospital environment is where a substantial increase has occurred in the last two decades. Patients with extensive abdominal surgery, indwelling devices, or who are undergoing procedures such as peritoneal dialysis are at greatest risk. Most infections are acquired from the endogenous flora but spread between patients has been documented. From 10 to 15% of all nosocomial urinary tract, intra-abdominal, and bloodstream infections are due to enterococci.

### PATHOGENESIS

Virulence factors are not known

Enterococci are a significant cause of disease in specialized hospital settings, but they are not highly virulent. On their own, they do not produce fulminant disease and in wound and soft tissue infections are usually mixed with other members of the intestinal flora. Some have even doubted their significance when isolated with more virulent members of the Enterobacteriaceae (see Chapter 21) or *Bacteroides fragilis* (see Chapter 19). Although some surface proteins are candidate adhesins, no virulence factors have been discovered.

## ENTEROCOCCAL DISEASE: CLINICAL ASPECTS

### MANIFESTATIONS

UTIs and soft tissue infections are most common

Enterococci cause opportunistic urinary tract infections (UTIs) and occasionally wound and soft tissue infections, in much the same fashion as members of the Enterobacteriaceae.

Infections are often associated with urinary tract manipulations, malignancies, biliary tract disease, and gastrointestinal disorders. Vascular or peritoneal catheters are often points of entry. Respiratory tract infections are rare. There is sometimes an associated bacteremia, which can result in the development of endocarditis on previously damaged cardiac valves.

## TREATMENT

The outstanding feature of the enterococci is their high and increasing levels of resistance to antimicrobial agents. Inherently relatively resistant to $\beta$-lactams and aminoglycosides, enterococci also have particularly efficient means of acquiring plasmid and transposon resistance genes from themselves and other species. All enterococci require 4 to 16 $\mu$g/mL of penicillin for inhibition due to decreased affinity of their penicillin-binding proteins for all $\beta$-lactams. Higher levels of resistance have been increasing, including the emergence of $\beta$-lactamase-producing strains, particularly in *E. faecalis*. The $\beta$-lactamase genes are identical to those in *Staphylococcus aureus*. Fortunately $\beta$-lactamase–producing strains have not yet become widely disseminated. Ampicillin remains the most consistently active agent against enterococci.

Inherent penicillin resistance is enhanced with $\beta$-lactamase emergence

Enterococci share with streptococci a relative resistance to aminoglycosides based on failure of the antimicrobic to be actively transported into the cell. Despite this, many strains of enterococci are inhibited and rapidly killed by combinations of low concentrations of penicillin and aminoglycosides. Under these conditions, the action of penicillin on the cell wall allows the aminoglycoside to enter the cell and act at its ribosomal site. Some strains show high level resistance to aminoglycosides based on mutations at the ribosomal binding site or the presence of aminoglycoside-inactivating enzymes. These strains do not demonstrate synergistic effects with penicillin.

Synergy between penicillin and aminoglycosides is based on access to ribosomes

Recently, resistance to vancomycin, the antibiotic most used for penicillin-resistant strains has emerged. Vancomycin resistance is due to a subtle change in peptidoglycan precursors, which are generated by ligases that modify the terminal amino acids of cross-linking acid side chains at the point at which $\beta$-lactams bind. The modifications decrease the binding affinity for penicillins 1000-fold without a detectable loss in peptidoglycan strength. Although hospitals vary, the average rate of resistance in enterococci isolated from intensive care units is around 20%. Enterococci are consistently resistant to sulfonamides and often resistant to tetracyclines, erythromycin, and cephalosporins.

Vancomycin resistance is emerging threat

Ligases modify peptidoglycan side chains

Penicillin or ampicillin remain the agents of choice for most UTIs and minor soft tissue infections. More severe infections, particularly endocarditis, are usually treated with combinations of a penicillin and aminoglycoside. If the strain fails to demonstrate penicillin–aminoglycoside synergism and/or is vancomycin resistant, some other combination guided by susceptibility testing must be selected.

Ampicillin or combinations of antimicrobics are used

## ADDITIONAL READING

Cunningham MW. Pathogenesis of group A streptococcal infections. *Clin Microbiol Rev* 2000;13:470–511. This scholarly review pays particular attention to pyrogenic exotoxins and newly discovered virulence factors.

Jedrzejas MJ. Pneumococcal virulence factors: structure and function. *Microbiol Mol Biol Rev* 2001;65:187–207. This review makes it clear that there is much more to the pneumococcus than its capsule.

Lancefield RC: A serological differentiation of human and other groups of hemolytic streptococci. *J Exp Med* 1933;57:571–595. This classic study changed streptococcal classification.

Schuchat A. Epidemiology of group B streptococcal disease in the United States: Shifting paradigms. *Clin Microbiol Rev* 1998;11:497–513. In addition to a very complete coverage of GBS disease, this review describes and evaluates the various strategies for prevention.

Schuchat A. Group B streptococcal disease: From trials and tribulations to triumph and trepidation. *Clin Infect Dis* 2001;33:751–756. This published lecture by the author of the above review gives an update on the prevention of GBS disease.

Stollerman GH. Rheumatic fever in the 21st century. *Clin Infect Dis* 2001;33:806–814. This brief review reads like a personal conversation with this seasoned veteran of the field.

CHAPTER 18

# *Corynebacterium, Listeria,* and *Bacillus*

KENNETH J. RYAN

This chapter includes a variety of highly pathogenic Gram-positive rods that are not currently common causes of human disease. Their medical importance lies in the lessons learned when they were more common, and the continued threat their existence poses. *Corynebacterium diphtheriae,* the cause of diphtheria, is a prototype for toxigenic disease. *Listeria monocytogenes* is a sporadic cause of meningitis and other infections in the fetus, newborn, and immunocompromised host. Occurrences in 2001 have served as a painful reminder that *Bacillus anthracis,* the cause of anthrax, is still the agent with the most potential for use in bioterrorism. The characteristics of these bacilli are presented in Table 18–1.

## CORYNEBACTERIA

Corynebacteria (from the Greek koryne, club) are small and pleomorphic. The genus *Corynebacterium* includes many species of aerobic and facultative Gram-positive rods. The cells tend to have clubbed ends, and often remain attached after division, forming "Chinese letter" or palisade arrangements. Spores are not formed. Growth is generally best under aerobic conditions on media enriched with blood or other animal products, but many strains will grow anaerobically. Colonies on blood agar are typically small (1 to 2 mm), and most are nonhemolytic. Catalase is produced, and many strains form acid (usually lactic acid) through carbohydrate fermentation.

Pleomorphic club-shaped rods grow on blood agar

### *Corynebacterium diphtheriae*

*C. diphtheriae* produces a powerful exotoxin that is responsible for diphtheria. Other corynebacteria are nonpathogenic commensal inhabitants of the pharynx, nasopharynx, distal urethra, and skin; they are collectively referred to as "diphtheroids." The species that have disease associations are included in Table 18–2.

*C. diphtheriae* produces exotoxin

Other corynebacteria are called diphtheroids

TABLE 18–1

**Features of Aerobic Gram-Positive Bacilli**

| Organism | Capsule | Endospores | Motility | Toxins | Source | Disease |
|---|---|---|---|---|---|---|
| *Corynebacterium diphtheriae* | – | – | – | DT | Human cases, carriers | Diphtheria |
| *Listeria monocytogenes* | – | – | + | LLO | Food, animals | Meningitis, bacteremia |
| *Bacillus* | | | | | | |
| *B. anthracis* | + | + | – | Exotoxin[a] | Imported animal products | Anthrax |
| *B. cereus* | – | + | + | Enterotoxin, pyogenic toxin | Ubiquitous | Food poisoning, opportunistic infection |
| Other species | – | + | + | | Ubiquitous | |

*Abbreviations:* DT, diphtheria toxin; LLO, listerolysin O.

[a] Exotoxin contains three components: lethal factor, protective antigen, and edema factor.

## BACTERIOLOGY

*C. diphtheriae* can produce DT coded by lysogenic phage

*C. diphtheriae* are differentiated from other corynebacteria by the appearance of colonies on the selective media used for its isolation and a variety of biochemical reactions. Strains of *C. diphtheriae* may or may not produce **diphtheria toxin (DT)**. Those that do have the structural gene for DT acquired from the genome of a specific bacteriophage. Only strains that are lysogenic for these phages produce toxin.

TABLE 18–2

**Other Aerobic and Facultative Gram-Positive Bacilli**

| Organism | Features | Epidemiology | Disease |
|---|---|---|---|
| *Corynebacterium ulcerans* | Closely related to *C. diphtheriae,* including ability to produce small amounts of DT | Similar to diphtheria, also infects animals | Pharyngitis |
| *Corynebacterium jeikeium* | Multiresistant, often susceptible only to vancomycin | Acquired from skin colonization | Bacteremia, IV catheter colonization |
| *Erysipelothrix rhusiopathiae* | Resembles corynebacteria and *Listeria* | Traumatic inoculation from animal and decaying organic matter | Erysipeloid, painful, slow-spreading, erythematous swelling of skin. Occupational disease of fishermen, butchers, and veterinarians |
| *Lactobacillus* spp. | Long, slender rods with squared ends, often chain end to end | Normal oral, gastrointestinal, and vaginal flora | No human infections *L. acidophilus* plays role in pathogenesis of dental caries |
| *Propionibacterium* | Resemble corynebacteria, anaerobes, or microaerophiles | Normal skin flora | Rare cause of bacterial endocarditis |

*Abbreviations:* DT, diphtheria toxin; IV, intravenous.

DT is an A-B toxin that acts in the cytoplasm to inhibit protein synthesis irreversibly in a wide variety of eukaryotic cells (Fig 18–1). After binding mediated by the B subunit, both the A and B subunits enter the cell in a endocytotic vacuole. In the low pH of the vacuole, the toxin unfolds exposing sites that facilitate translocation of the A subunit from the phagosome to the cytosol. Separation is required for full activity of the A subunit on its target protein elongation factor 2 (EF-2), which transfers polypeptidyl-transfer RNA from acceptor to donor sites on the ribosome of the host cell. The specific action of the A subunit is to catalyze the transfer of the adenine ribose phosphate portion of nicotinamide adenine dinucleotide (NAD) to EF-2, an enzymatic reaction called **ADP-ribosylation.** This inactivates EF-2 and shuts off protein synthesis. The ADP-ribosylation leaves the toxin itself free to catalyze another reaction, making it possible for a single DT molecule to inhibit protein synthesis in a cell within a few hours. ADP-ribosylation is now known to be the enzymatic mechanism of action for a number of toxins including those that act on EF-2 (DT, *Pseudomonas aeruginosa* exotoxin A) and those with other target proteins (cholera toxin, *Escherichia coli* LT, pertussis toxin). *C. diphtheriae* itself is unaffected because it uses a protein other than EF-2 in protein synthesis.

A subunit enters the cytosol from a vacuole

EF-2 is inactivated by ADP-ribosylation

Transfer of tRNA and protein synthesis are stopped

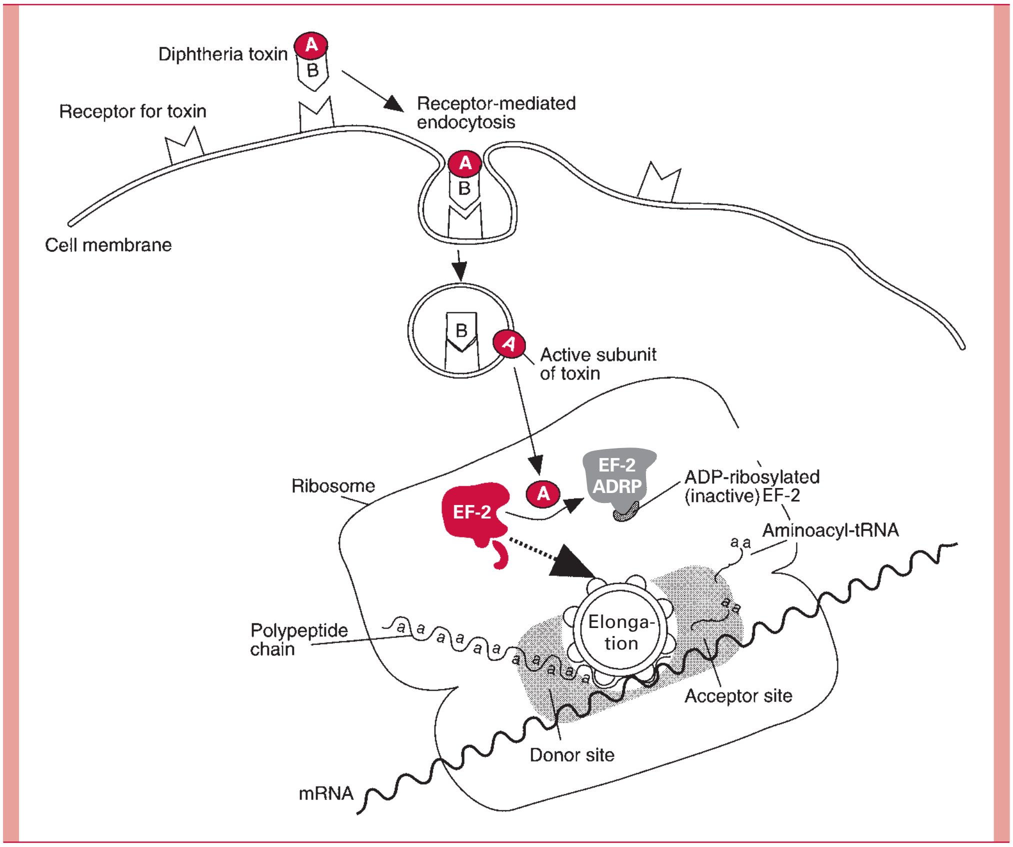


FIGURE 18–1

Action of diphtheria toxin. The toxin-binding (B) portion attaches to the cell membrane, and the complete molecule enters the cell. In the cell, the A subunit dissociates and catalyzes a reaction that ADP-ribosylates and thus inactivates elongation factor 2 (EF-2). This factor is essential for ribosomal reactions at the acceptor and donor sites, which transfer triplet code from messenger RNA (mRNA) to amino acid sequences via transfer RNA (tRNA). Inactivation of EF-2 stops building of the polypeptide chain.

## DIPHTHERIA

CLINICAL CAPSULE

Diphtheria is a disease caused by the local and systemic effects of diphtheria toxin, a potent inhibitor of protein synthesis. The local disease is a severe pharyngitis typically accompanied by a plaquelike pseudomembrane in the throat and trachea. The life-threatening aspects of diphtheria are due to the absorption of the toxin across the pharyngeal mucosa and its circulation in the bloodstream. Multiple organs are affected, but the most important is the heart, where the toxin produces an acute myocarditis.

### EPIDEMIOLOGY

Transmitted by respiratory droplets

Most cases are in unimmunized transients

*C. diphtheriae* is transmitted by droplet spread, by direct contact with cutaneous infections, and, to a lesser extent, by fomites. Some subjects become convalescent pharyngeal or nasal carriers and continue to harbor the organism for weeks to months or even for a lifetime. Diphtheria is rare where immunization is widely used. In the United States, for example, fewer than 10 cases are now reported each year. These usually occur as small outbreaks in populations that have not received adequate immunization, such as migrant workers, transients, and those who refuse immunization on religious grounds. It has been more than 20 years since any outbreak exceeded 50 cases.

Outbreaks occur when immunization rates decrease

Diphtheria still occurs in developing countries and in those places where public health infrastructure has been disrupted. For example, in the former Soviet Union, where the annual number of diphtheria cases had been below 200, over 47,000 cases and 1700 deaths occurred between 1990 and 1995. This outbreak followed the introduction of diphtheria into a population where the immunization rate for children was not sufficiently high, adults were not given boosters, and the efforts at mass immunization early in the epidemic were inadequate.

### PATHOGENESIS

A subunit inhibits protein synthesis

B-subunit binding determines cell susceptibility

*C. diphtheriae* has little invasive capacity, and diphtheria is due to the local and systemic effects of DT, a protein exotoxin with potent cytotoxic features. It inhibits protein synthesis in cell-free extracts of virtually all eukaryotic cells, from protozoa and yeasts to higher plants and humans. Its toxicity for intact cells varies among mammals and organs, primarily as a result of differences in toxin binding and uptake. In humans the B subunit binds to one of a common family of eukaryotic receptors that regulate cell growth and differentiation, thus exploiting a normal cell function.

Local effects produce pseudomembrane

Absorption of DT leads to myocarditis

The production of DT has both local and systemic effects. Locally, its action on epithelial cells leads to necrosis and inflammation, forming a pseudomembrane composed of a coagulum of fibrin, leukocytes, and cellular debris. The extent of the pseudomembrane varies from a local plaque to an extensive covering of much of the tracheobronchial tree. Absorption and circulation of DT allows binding throughout the body. Myocardial cells are most affected; eventually, acute myocarditis develops.

Iron concentration modulates toxin and other correlates of virulence

Nontoxigenic strains produce mild disease

The DT **tox gene** is regulated by a repressor protein (DtxR) in response to iron limitation. Toxin biosynthesis is greatest when the bacteria are grown at low iron concentrations. Iron seems to play a central role in the expression of virulence; the repressor also regulates a corynebacterial siderophore system and a number of other proteins. Nontoxigenic strains of *C. diphtheriae* can produce pharyngitis, but not the toxic manifestations of diphtheria. They can be converted to toxigenicity by lysogenization in vitro with phage, and this process can probably occur in vivo.

### IMMUNITY

Antibodies neutralize toxin

Toxoid is formalin inactivated DT

Diphtheria toxin is antigenic, stimulating the production of protective antitoxin antibodies during natural infection. Formalin treatment of toxin produces **toxoid,** which retains the antigenicity but not the toxicity of native toxin and is used in immunization against the disease. It is clear that this process functionally inactivates fragment B. Whether it also inactivates fragment A or prevents its ability to dissociate from fragment B is not known. Molecular studies of the A subunit structure and action suggest that another approach to immunization may be through genetic engineering. For example, substitution of a single amino acid located in the NAD-binding site of the A subunit of DT can completely detoxify but retain the immunogenic specificity of the toxin. The membrane-translocation properties of the B subunit have also been used to transport other proteins into the cytosol by linking them to DT.

# DIPHTHERIA: CLINICAL ASPECTS

## MANIFESTATIONS

After an incubation period of 2 to 4 days, diphtheria usually presents as pharyngitis or tonsillitis. Typically, malaise, sore throat, and fever occur, and a patch of exudate or membrane develops on the tonsils, uvula, soft palate, or pharyngeal wall. The gray-white pseudomembrane adheres to the mucous membrane, and may extend from the oropharyngeal area down to the larynx and into the trachea. Associated cervical adenitis is common, and in severe cases cervical adenitis and edema produce a "bullneck" appearance. In uncomplicated cases, the infection gradually resolves, and the membrane is coughed up after 5 to 10 days.

Severe pharyngitis may have exudate or membrane

The complications and lethal effects of diphtheria are caused by respiratory obstruction or by the systemic effect of DT absorbed at the site of infection. Mechanical obstruction of the airway produced by the pseudomembrane, edema, and hemorrhage can be sudden and complete and can lead to suffocation, particularly if large sections of the membrane separate from the tracheal or laryngeal epithelial surface. The DT absorbed into the circulation causes injury to various organs, most seriously the heart. Diphtheritic myocarditis appears during the second or third week in severe cases of respiratory diphtheria. It is manifested by cardiac enlargement and weakness, arrhythmia, and congestive heart failure with dyspnea. Nervous system involvement appears later in the course of disease, most often involving paralysis of the soft palate, oculomotor (eye) muscles, or select muscle groups. The paralysis is reversible and is generally not serious unless the diaphragm is involved. The disease resolves with the formation of antitoxin antibody.

Pseudomembrane can block the airway

DT myocarditis may lead to congestive heart failure

*C. diphtheriae* may produce nonrespiratory infections, particularly of the skin. The characteristic lesion, which ranges from a simple pustule to a chronic, nonhealing ulcer, is most common in tropical and hot, arid regions. Cardiac and neurologic complications from these infections are infrequent, suggesting that the efficiency of toxin production or absorption is low compared to that in respiratory infections.

Cutaneous diphtheria produces ulcerative lesion

## DIAGNOSIS

The initial diagnosis of diphtheria is entirely clinical. There are presently no rapid laboratory tests of sufficient value to influence the decision regarding antitoxin administration. Direct smears of infected areas of the throat are not reliable diagnostic tools. Definitive diagnosis is accomplished by isolating and identifying *C. diphtheriae* from the infected site and demonstrating its toxigenicity. Isolation is usually achieved with a selective medium containing potassium tellurite (eg, Tinsdale medium).

Primary diagnosis is clinical

Culture requires special medium

It should be recognized that while the diagnosis of diphtheria could be once be made and confirmed with great confidence, it is now more difficult because experience with the disease is rare. Most physicians have never seen a case of diphtheria, and most laboratories have never isolated the organism and do not even stock the required medium. Because routine throat culture procedures will not detect *C. diphtheriae,* the physician must advise the laboratory of the suspicion of diphtheria in advance. Generally, 2 days are required to exclude *C. diphtheriae* (ie, no colonies isolated on Tinsdale agar); however, more time is needed to complete identification and toxigenicity testing of a positive culture.

Laboratory must be notified of suspicion in advance

## TREATMENT

Treatment of diphtheria is directed at neutralization of the toxin with concurrent elimination of the organism. The former is most critical and is accomplished by promptly administering a

Antitoxin therapy aimed at neutralizing free toxin

Erythromycin most effective antimicrobic therapy

diphtheria antitoxin that neutralizes free toxin, but it will have no effect on toxin already fixed to cells. *C. diphtheriae* is susceptible to a variety of antimicrobics, including penicillins, cephalosporins, erythromycin, and tetracycline. Of these, erythromycin has been the most effective. The complications of diphtheria are managed primarily by supportive measures.

## PREVENTION

The mainstay of diphtheria prevention is immunization. The vaccine is highly effective. Three to four doses of diphtheria toxoid produce immunity by stimulating antitoxin production. The initial series is begun in the first year of life (see Chapter 12). Booster immunizations at 10-year intervals maintain immunity. Fully immunized individuals may become infected with *C. diphtheriae*, because the antibodies are directed only against the toxin, but the disease is mild. Serious infection and death occur only in unimmunized or incompletely immunized individuals. Immunization with DT toxoid prevents serious toxin-medicated disease.

# *LISTERIA MONOCYTOGENES*

## BACTERIOLOGY

Rods resemble corynebacteria

Colonies are $\beta$ hemolytic

*Listeria monocytogenes* is a Gram-positive rod with some bacteriologic features that resemble those of both corynebacteria and streptococci. In stained smears of clinical and laboratory material, the organisms resemble diphtheroids. *Listeria* are not difficult to grow in culture, producing small, $\beta$-hemolytic colonies on blood agar. This species is able to grow slowly in the cold even at temperatures as low as 1°C. *Listeria* species are catalase positive, which distinguishes them from streptococci, and produce a characteristic tumbling motility in fluid media at 25°C that distinguishes them from corynebacteria.

Pathogenic serotypes have unique teichoic acid

Eleven *L. monocytogenes* serotypes are recognized based on flagellar and somatic surface antigens, but the majority of human cases are limited to only three serotypes (1/2a, 1/2b, 4b). These serotypes differ from other *Listeria* in elements of the chemical teichoic acid composition, a major component of their cell wall. The teichoic acid of serotype 4b, which accounts for almost all food-borne listeriosis outbreaks, is distinctive in that there are both galactose and glucose substituents in its *N*-acetylglucosamine.

## LISTERIOSIS

CLINICAL CAPSULE

Listeriosis is often an insidious infection in humans. Infection of the fetus or newborn may result in stillbirth or a fulminant neonatal sepsis. In most adults, there are usually only general manifestations, such as fever and malaise, associated with bacteremia.

## EPIDEMIOLOGY

Reservoir is intestine of animals and humans

Food-borne transmission is from animal products

Members of *Listeria* are widespread among animals in nature, including those associated with our food supply (eg, fowl, ungulates). The human reservoir appears to be intestinal colonization, which various studies have shown to range from 2 to 12%. The importance of food-borne transmission of listeriosis was not recognized until the early 1980s. A widely publicized 1985 California outbreak involved consumption of Mexican-style soft cheese and included 86 cases and 29 deaths. Most of the cases were among mother–infant pairs. Dairy product outbreaks have been traced to post-pasteurization contamination or deviation from recommended time and temperature guidelines. An important feature of some epidemics has been the ability of *L. monocytogenes* to grow at refrigerator temperatures, allowing scant numbers to reach an infectious dose during storage. Heightened awareness has implicated many other foodstuffs, particularly those prepared from animal products in a ready-to-eat form such as sausages and delicatessen poultry items.

Cold growth enhances infectivity

*L. monocytogenes* may also be transmitted transplacentally to the fetus, presumably following hematogenous dissemination in the mother. It may also be transmitted to newborns in the birth canal in a manner similar to group B streptococci. Listeriosis is still not a reportable disease in the United States, but active surveillance studies indicate that it may account for more than 1000 cases and 200 deaths each year. Most cases occur at the extremes of life (eg, in infants less than 1 month of age or adults over 60 years of age).

Transplacental and birth canal transmission can occur

## PATHOGENESIS

*L. monocytogenes* animal models have long been used for the study of cell-mediated immunity because of the ability of the organism to grow in nonimmune macrophages. An activated macrophage is needed to clear the infection, and in fact the concept of the activated macrophage that appears throughout this book owes much to the study of experimental *Listeria* infection. More recently, *Listeria* has generated great interest because of the mechanisms it uses to invade and survive in macrophages and efficiently spread among epithelial cells.

Grows in nonimmune macrophages

The first step in this process takes place when *L. monocytogenes* attaches to and is internalized into nonprofessional and professional phagocytes. These include enterocytes, fibroblasts, dendritic cells, hepatocytes, endothelial cells, M cells, and macrophages. Under the influence of a surface protein called **internalin,** *Listeria* causes a local reorganization of the cytoskeleton of the cell and stimulates its own entry in a membrane-bound vacuole. The invading bacteria rapidly escape into the host cell cytosol by elaborating **listeriolysin O** (LLO), which acts in a manner similar to streptolysin O and other pore-forming cytotoxins.

Surface internalin starts epithelial cell invasion

Enters cell in vacuole

LLO aids escape to cytosol

Once in the cytosol, *L. monocytogenes* continues to move through the cell by controlling the metabolism of the cell's actin filaments. This process is stimulated by other surface proteins (ActA, gelsolin), which control the actin polymerization so that actin monomers are sequentially concentrated directly behind the bacterium. The net effect is the appearance of a bacterial "tail" that is connected to the long actin filaments. The addition of new actin units to the tail propels the organisms through the cytosol like a comet through the evening sky (Fig 18–2). The motile *Listeria* eventually reach the edge of the cell where, instead of stopping, they protrude into the adjacent cell taking the original cell membrane along with them. When these pinch off, the organisms are surrounded by a

Actin polymerization forms motile comet tails

Protrudes into adjacent cell

LLO releases bacteria again

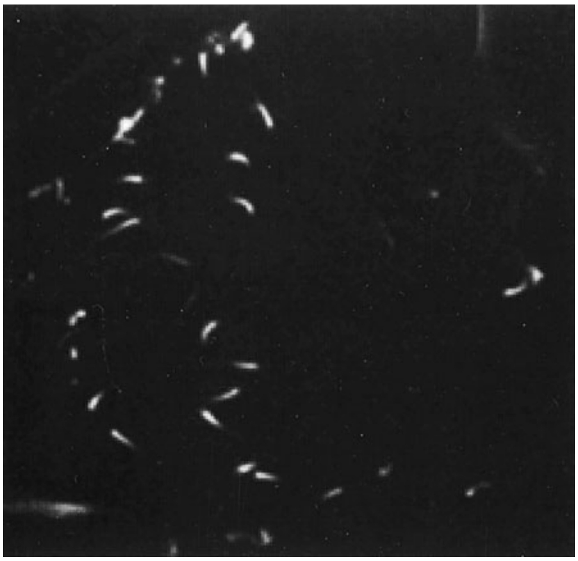

FIGURE 18–2
Intracellular movement of *Listeria monocytogenes. L. monocytogenes* cells are shown within infected cells in culture. The immunofluorescent stain used an antibody that binds to actin, demonstrating the comet-like actin "tails," which trail the bacteria as they move through the cell. (*From Niebuhr K, Chakraborty T, Rohde M, et al:* Infect Immun *1993;61:2793–2802, Figure 4a, with permission.*)

double set of host cell membranes that are dissolved by LLO and phospholipases, releasing the organisms to start the cycle again.

Cell-to-cell spread avoids the immune system

Multiple virulence factors are regulated together

This complex strategy allows *L. monocytogenes* to survive in macrophages by escaping the phagosome, and then to spread from epithelial cell to epithelial cell without exposure to the immune system. How does *Listeria* keeps its LLO from destroying the host cell membrane from the inside as the pore-forming toxins of other bacteria do from the outside? It appears that *L. monocytogenes* may be able to not only regulate the timely production of LLO but also to trigger its degradation by host cell proteolytic enzymes after it has left the endosome vacuole. LLO and several other genes, including those involved in actin rearrangement, are all part of a virulence regulon. The result is a surgically precise deployment of virulence factors.

## IMMUNITY

*Listeria*-specific T-cell activation protects

Immunity to *Listeria* infection owes little to humoral and much to cell-mediated mechanisms. The generation of antigen-specific CD4+ and CD8+ T-cell subsets is required for the resolution of infection and the establishment of long-lived protection. Neutrophils play a role in early stages by lysing *Listeria* infected cells, but it is cytokine-activation that reverses the intracellular growth in macrophages. The importance of cellular immunity is emphasized by the increased frequency of listeriosis in those with its compromise due to disease such as acquired immunodeficiency syndrome (AIDS), immunosuppressive therapy, age, or pregnancy.

# LISTERIOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

Bacteremia is usually occult

Meningitis and encephalitis are produced

Listeriosis usually does not present clinically until there is disseminated infection. In foodborne outbreaks, sometimes gastrointestinal manifestations of primary infection such as nausea, abdominal pain, diarrhea, and fever occur. Disseminated infection in adults is usually occult, involving fever, malaise, and constitutional symptoms without an obvious focus. *L. monocytogenes* has a tropism for the central nervous system (CNS), including the brain parenchyma (encephalitis) and brainstem, but the meningitis it causes is not distinct from that associated with other leading bacterial pathogens (*Streptococcus pneumoniae, Neisseria meningitidis*).

Puerperal infection leads to stillbirth and dissemination

Incidence in AIDS is greatly increased

Neonatal and puerperal infections appear in settings similar to those of infections with group B streptococci. Intrauterine infection leads to stillbirth or a disseminated infection at or near birth. If the pathogen is acquired in the birth canal, the onset of disease is later. The risk of disease is increased in elderly and immunocompromised individuals as well as women in late pregnancy. The number of cases in AIDS patients has been estimated at 300 times the general population.

## DIAGNOSIS

Blood and CSF culture reveals Gram-positive rods

Diagnosis of listeriosis is by culture of blood, cerebrospinal fluid (CSF), or focal lesions. In meningitis, CSF Gram stains are usually positive. The first indication that *Listeria* is involved is often the discovery that the $\beta$-hemolytic colonies subcultured from a blood culture bottle are Gram-positive rods rather than streptococci.

## TREATMENT AND PREVENTION

Penicillins and TMP-SMX are effective

*L. monocytogenes* is susceptible to penicillin G, ampicillin, and trimethoprim/sulfamethoxazole, all of which have been used effectively. Ampicillin combined with gentamicin is considered the treatment of choice for fulminant cases. Intense surveillance to prevent the sale of *Listeria*-contaminated ready-to-eat meat products has led to a marked decrease in the incidence of new infections. There is no vaccine.

## BACILLUS

The genus *Bacillus* includes many species of aerobic or facultative, spore-forming, Gram-positive rods. With the exception of one species, *B. anthracis,* they are low-virulence saprophytes widespread in air, soil, water, dust, and animal products. *B. anthracis* causes the zoonosis anthrax, a disease of animals that is occasionally transmitted to humans.

The genus is made up of rod-shaped organisms that can vary from coccobacillary to rather long-chained filaments. Motile strains have peritrichous flagella. Formation of round or oval spores, which may be central, subterminal, or terminal depending on the species, is characteristic of the genus. *Bacillus* species are Gram positive; however, positivity is often lost, depending on the species and the age of the culture.

Gram-positive spore-forming rods

With *Bacillus,* growth is obtained with ordinary media incubated in air and is reduced or absent under anaerobic conditions. The bacteria are catalase positive and metabolically active. The spores survive boiling for varying periods and are sufficiently resistant to heat that those of one species are used as a biologic indicator of autoclave efficiency. Spores of *B. anthracis* survive in soil for decades.

Aerobic conditions preferred for growth

Heat-resistant spores survive boiling

## *Bacillus anthracis*

### BACTERIOLOGY

*B. anthracis* has a tendency to form very long chains of rods and in culture is nonmotile and nonhemolytic; colonies are characterized by a rough, uneven surface with multiple curled extensions at the edge resembling a “Medusa head.” *B. anthracis* has a D-glutamic acid polypeptide capsule of a single antigenic type that has antiphagocytic properties. The organism is also is a potent producer of one or more exotoxins, which although they have been given multiple names (lethal factor, edema factor, protective antigen), represent separate activities of a protein complex. In various combinations and configurations these proteins may exhibit binding, cytolytic, or enzymatic activity. One such combination exhibits adenylate cyclase activity similar to that seen in *Bordetella pertussis* (see Chapter 24).

Chained rods and “Medusa head” colonies are typical

Polypeptide capsule is antiphagocytic

Exotoxin complex has multiple actions

### ANTHRAX

CLINICAL CAPSULE

Human anthrax is typically an ulcerative sore on an exposed part of the body. Constitutional symptoms are minimal, and the ulcer usually resolves without complications. If anthrax spores are inhaled, a fulminant pneumonia may lead to respiratory failure and death.

The isolation of *B. anthracis,* the proof of its relationship to anthrax infection, and the demonstration of immunity to the disease are among the most important events in the history of science and medicine. Robert Koch rose to fame in 1877 by growing the organism in artificial culture using pure culture techniques. He defined the stringent criteria needed

Pasteur produced animal vaccine with attenuated anthrax strain to prove that the organism caused anthrax (Koch's postulates), then met them experimentally. Louis Pasteur made a convincing field demonstration at Pouilly-le-Fort to show that vaccination of sheep, goats, and cows with an attenuated strain of *B. anthracis* prevented anthrax. He was cheered and carried on the shoulders of the grateful farmers of the district, an experience now, unhappily, largely restricted to winning football coaches.

## EPIDEMIOLOGY

Infection is through injection of spores derived from herbivores into the skin

Contaminated materials are imported from countries with animal anthrax

Anthrax is primarily a disease of herbivores such as horses, sheep, and cattle who acquire it from spores of *B. anthracis* contaminating their pastures. Humans become infected through contact with these animals or their products in a way that allows the spores to be inoculated through the skin, ingested, or inhaled. In the 1920s, more than 100 cases occurred annually in the United States among farmers, veterinarians, and meat handlers, but the control of animal anthrax in developed countries has made human cases rare. A few endemic foci persist in North America and have been the source of naturally acquired disease. Another source is animal products such as wool, hides, or bone meal fertilizer that have been imported from a country where animal anthrax is endemic.

Use for biological warfare is a continuing threat

Aerosols could spread pulmonary anthrax widely

Weapons-grade spores are specially treated

The real threat associated with anthrax comes from its continuing appeal to those bent on using it as an agent of biological warfare or terrorism. The long life, stability, and low mass of the dried spores make the prospect of someone producing a "cloud of death" leading to massive pulmonary anthrax a chilling reality. A 1979 episode resulting in more than 50 anthrax deaths in the former Soviet Union is now attributed to an accidental explosion at a biological warfare research facility that involved more than 20 pounds of anthrax spores. United Nations inspection teams in the Middle East recently uncovered facilities for the production of massive amounts of spores together with plans to create and spread infectious aerosols using missile warheads. The inhalation anthrax among postal workers following the September 11, 2001 terrorist attacks appears to have been due to the mailing of envelopes containing "weapons-grade" anthrax spores stolen from a biologic warfare research facility. Such spores had been treated to enhance their aerosolization and dissemination.

## PATHOGENESIS

Antiphagocytic effect of D-glutamic acid capsule is required for virulence

Exotoxins have multiple activities

When spores of *B. anthracis* reach the rich environment of human tissues, they germinate and multiply in the vegetative state. The antiphagocytic properties of the capsule aid in survival, eventually allowing production of large enough amounts of the exotoxins to cause disease. The tripartite nature of the anthrax exotoxin complex must play an important role but the timing and relative importance of the components are not known. The adenylate cyclase activity is believed to correlate with the striking edema seen at infected sites.

## IMMUNITY

Immune mechanisms are unknown

The specific mechanisms of immunity against *B. anthracis* are not known. Experimental evidence favors antibody directed against the toxin complex, but the relative role of the components of the toxin is not clear. The capsular glutamic acid is immunogenic but antibody against it is not protective.

## ANTHRAX: CLINICAL ASPECTS

Initial papule evolves to malignant pustule

Cutaneous anthrax usually begins 2 to 5 days after inoculation of spores into an exposed part of the body, typically the forearm or hand. The initial lesion is an erythematous papule, which may be mistaken for an insect bite. This papule usually progresses through vesicular and ulcerative stages in 7 to 10 days to form a black eschar (scab) surrounded by edema. This lesion complex is known as the "malignant pustule," although it is neither malignant nor a pustule. Associated systemic symptoms are usually mild, and the lesion typically heals very slowly after the eschar separates. Less commonly, the disease progresses with massive local edema, toxemia, and bacteremia; it has a fatal outcome if untreated.

Pulmonary anthrax is contracted by inhalation of spores. Historically, this occurred when contaminated hides, hair, wool, and the like are handled in a confined space (woolsorter's disease) or following laboratory accidents. Today it is the form we would expect from the dissemination of a spore aerosol in biologic warfare. In the pulmonary syndrome, 1 to 5 days of nonspecific malaise, mild fever, and nonproductive cough lead to progressive respiratory distress and cyanosis. Massive spread to the bloodstream and CNS follow rapidly. Mediastinal edema was a prominent finding in the postal workers. If untreated, progression to a fatal outcome is usually very rapid once bacteremia has developed.

Pulmonary anthrax is acquired by inhaling spores

Fever and cough progress to cyanosis and death

## DIAGNOSIS

Culture of skin lesions, sputum, blood, and CSF are the primary means of anthrax diagnosis. Given some suspicion on epidemiologic grounds, Gram stains of sputum or other biologic fluids showing large numbers of Gram-positive bacilli can indicate the diagnosis. In September of 2001, diagnosis of the first case in Florida was speeded by an infectious disease specialist who knew such rods were extremely rare in the spinal fluid. Such bacilli are also unusual in sputum.

Smears with large Gram-positive rods are suggestive

*B. anthracis* and other *Bacillus* species are not difficult to grow. In fact, clinical laboratories frequently isolate the nonanthrax species as environmental contaminants. The saprophytic species are usually $\beta$-hemolytic and motile; these features can be used to exclude *B. anthracis.* Blood cultures are positive in most cases of pulmonary anthrax.

Hemolysis and motility exclude *B. anthracis*

Sputum and blood cultures are positive in pneumonia

## TREATMENT

Antimicrobial treatment has little effect on the course of cutaneous anthrax but does protect against dissemination. Almost all strains of *B. anthracis* are susceptible to penicillin, which remains the treatment of choice for all forms of anthrax. Doxycycline or ciprofloxacin are alternatives and are also recommended for chemoprophylaxis in the case of known or suspected exposure.

Penicillin is the recommended treatment

Ciprofloxacin or doxycycline is used for prophylaxis

## PREVENTION

The most important preventive measures are those that eradicate animal anthrax and limit imports from endemic areas. Vaccines are also useful. Pasteur's vaccine used a live strain attenuated by repeated subculture that resulted in the loss of a plasmid encoding toxin production. A similar live vaccine is still effective for animals, but inactivated human vaccines have a less certain efficacy. The vaccine used by the US military is prepared from filtrates of a nonencapsulated *B. anthracis* strain that produces the protective antigen component of the toxin complex. Its acceptance is complicated by fears that the architects of biological warfare may have crafted strains for which this vaccine is not protective. Proof of the efficacy of the vaccine in humans is neither practical nor ethical.

Eradication of animal anthrax is most important

Live and inactivated vaccines are available but controversial

# *Other Bacillus Species*

*Bacillus* spores are widespread in the environment, and isolation of one of the more than 20 *Bacillus* species other than *B. anthracis* from clinical material usually represents contamination of the specimen. Occasionally *B. cereus, B. subtilis,* and some other species produce genuine infections, including infections of the eye, soft tissues, and lung. Infection is usually associated with immunosuppression, trauma, an indwelling catheter, or contamination of complex equipment such as an artificial kidney. The relative resistance of *Bacillus* spores to disinfectants aids their survival in medical devices that cannot be heat sterilized.

Spores enhance survival in medical devices

*B. cereus* produces pyogenic toxin and enterotoxin

*B. cereus* deserves special mention. This species is most likely to cause opportunistic infection, which suggests a virulence intermediate between that of *B. anthracis* and the other species. A strain isolated from an abscess has been shown to produce a destructive pyogenic toxin. *B. cereus* can also cause food poisoning by means of enterotoxins. One enterotoxin acts by stimulating adenyl cyclase production and fluid excretion in the same manner as toxigenic *E. coli* and *Vibrio cholerae* (see Chapters 21 and 22).

## ADDITIONAL READING

Aureli P, Fiorucci GC, Caroli D, Marchiaro G, Novara O, Leone L, Salmaso S. An outbreak of gastroenteritis associated with corn contaminated by *Listeria monocytogenes. N Engl J Med* 2000;342:1236–1241. This carefully studied outbreak in schools in adjacent Italian towns gives the best indication of the clinical findings in primary *Listeria* infection.

Dixon TC, Meselson M, Gillemin J, Hanna PC. Anthrax. *N Engl J Med* 1999; 341:815–826. This comprehensive review considers pathogenesis and clinical aspects.

Lorber B. Listeriosis. *Clin Infect Dis* 1997;24:1–11. A state-of-the-art review of the epidemiologic, clinical, and therapeutic aspects of the disease.

Mayer TA, et al. Clinical presentation of inhalation anthrax following bioterrorism exposure. *JAMA* 2001;286:2549–2553. This paper gives a detailed account of anthrax in two postal workers, including Gram stains, radiographs, and CT scans; these patients survived. (The paper that follows describes two fatal cases.)

McCloskey RV, Eller JJ, Green M, et al. The 1970 epidemic of diphtheria in San Antonio. *Ann Intern Med* 1970;75:495–503. A clear and informative description of a diphtheria outbreak is provided. The clinical features are given in detail, including color photographs of diphtheritic membranes.

Schlech WF, Lavigne PM, Bortolussi RA, et al. Epidemic listeriosis—evidence for transmission by food. *N Engl J Med* 1983;308:203–206. This epidemiologic study nicely traces events beginning on a Halifax farm to 34 cases of listeriosis. This outbreak was the first evidence that *Listeria* was a food-borne pathogen.

Southwick FS, Purich DL. Intracellular pathogenesis of listeriosis. *N Engl J Med* 1996;334:770–776. For a well-illustrated explanation of how *Listeria* uses the actin metabolism of the cell to make comet "tails," be sure to read this paper.

Vazquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Dominguez-Bernal G, et al. *Listeria* pathogenesis and molecular virulence determinants. *Clin Microbiol Rev* 2001;14:584–640. This extensive review covers all topics from disease in animals to molecular genetics.

Vitek C, Warton M. Diphtheria in the former Soviet Union: Reemergence of a pandemic disease. *Emerg Infect Dis* 1998;4:539–550. A concise summary of the problems and attempted solutions to the major diphtheria outbreak of the last quarter century.

CHAPTER 19

# *Clostridium, Peptostreptococcus, Bacteroides,* and Other Anaerobes

KENNETH J. RYAN

The bacteria discussed in this chapter are united by a common requirement for anaerobic conditions for growth. Organisms from multiple genera and all Gram stain categories are included. Most of them produce endogenous infections adjacent to the mucosal surfaces, where they are members of the normal flora. The clostridia form spores that allow them to produce diseases, such as tetanus and botulism, following environmental contamination of tissues or foods. Another anaerobic genus of bacteria, *Actinomyces,* is discussed in Chapter 29.

## GENERAL FEATURES: ANAEROBES AND ANAEROBIC INFECTION

## BACTERIOLOGY: ANAEROBIC BACTERIA

### THE NATURE OF ANAEROBIOSIS

Anaerobes not only survive under anaerobic conditions, they require them to initiate and sustain growth. By definition, anaerobes fail to grow in the presence of 10% oxygen, but some are sensitive to oxygen concentrations as low as 0.5% and are killed by even brief exposures to air. However, **oxygen tolerance** is variable, and many organisms can survive in 2 to 8% oxygen, including most of the pathogenic species. The mechanisms involved are incompletely understood but clearly represent a continuum from species described as **aerotolerant** to those that require the culture medium to be prepared and stored under anaerobic conditions.

Anaerobes require low oxygen to initiate growth

Oxygen tolerance is a continuum

Anaerobes lack the cytochromes required to use oxygen as a terminal electron acceptor in energy-yielding reactions, and thus generate energy solely by fermentation (see Chapter 3). Some anaerobes will not grow unless the oxidation-reduction potential is

Low redox potential is required

extremely low (−300 mV), because critical enzymes must be in the reduced state to be active, aerobic conditions create a metabolic block.

Defense against oxygen products is lacking

Pathogens often have catalase and superoxide dismutase

Another element of anaerobiosis is the direct susceptibility of anaerobic bacteria to oxygen. For most aerobic and facultative bacteria, **catalase** and/or **superoxide dismutase** neutralize the toxicity of the oxygen products **hydrogen peroxide** and **superoxide** (see Chapter 3). Most anaerobes lack these enzymes and are injured when these oxygen products are formed in their microenvironment. As will be discussed below, some of the most virulent anaerobic pathogens are able to produce catalase or superoxide dismutase.

## CLASSIFICATION

Biochemical, cultural, and molecular criteria define many species

The anaerobes indigenous to humans include almost every morphotype and hundreds of species. Typical biochemical and cultural tests are used for classification, although this is difficult because the growth requirements of each anaerobic species must be satisfied. Characterization of cellular fatty acids and metabolic products by chromatography has been useful for many anaerobic groups. Nucleic acid base composition and homology have been used extensively to rename older taxonomy. The genera most commonly associated with disease are shown in Table 19–1 and discussed below.

### Anaerobic Cocci

Gram(+) = *Peptostreptococcus*

Gram(−) = *Veillonella*

Virtually all the medically important species of anaerobic Gram-positive cocci are now classified in a single genus, ***Peptostreptococcus.*** With Gram staining, these bacteria are most often seen as long chains of tiny cocci. ***Veillonella,*** a Gram-negative genus, deserves mention because of its potential for confusion with *Neisseria,* the only other Gram-negative coccus (see Chapter 20).

### Clostridia

The clostridia are large, spore-forming, Gram-positive bacilli. Like their aerobic counterpart, *Bacillus,* clostridia have spores that are resistant to heat, desiccation, and disinfectants. They are able to survive for years in the environment and return to the vegetative form when placed in a favorable milieu. The shape of the cell and location of the spore varies with the species, but the spores themselves are rarely seen in clinical specimens.

Spores vary in shape and location

Hemolysin, neurotoxin, and enterotoxin production cause disease

The medically important clostridia are potent producers of one or more protein exotoxins. The histotoxic group including ***Clostridium perfringens*** and five other species (see Table 19–2) produces hemolysins at the site of acute infections that have lytic effects on a wide variety of cells. The neurotoxic group including ***C. tetani*** and ***C. botulinum*** produces neurotoxins that exert their effect at neural sites remote from the bacteria. ***C. difficile*** produces enterotoxins and disease in the intestinal tract. Many of the more than 80 other nontoxigenic clostridial species are also associated with disease.

TABLE 19–1

**Usual Locations of Opportunistic Anaerobes**

| Organism | Gram Stain | Mouth or Pharynx | Intestine | Urogenital Tract | Skin |
|---|---|---|---|---|---|
| *Peptostreptococcus* | Positive cocci | + | + | + | − |
| *Propionibacterium* | Positive rods | − | − | − | + |
| *Clostridium* | Positive rods (large) | − | + | − | − |
| *Bacteroides fragilis* group | Negative rods (coccobacillary) | − | + | − | − |
| *Fusobacterium* | Negative rods (elongated) | + | + | − | − |
| *Prevotella* | Negative rods | + | | + | − |
| *Porphyromonas* | Negative rods | + | | + | |

TABLE 19–2

**Features of Pathogenic Anaerobes**

| Organism | Bacteriologic Features | Exotoxins | Source | Disease |
|---|---|---|---|---|
| **Gram-positive cocci** | | | | |
| *Peptostreptococcus* | | | Mouth, intestine | Oropharyngeal infections, brain abscess |
| **Gram-negative cocci** | | | | |
| *Veillonella* | | | Intestine | Rare opportunist |
| **Gram-positive bacilli** | | | | |
| *Clostridium perfringens* | Spores | $\alpha$-toxin, $\theta$-toxin, enterotoxin | Intestine, environment, food | Cellulitis, gas gangrene, enterocolitis |
| Histotoxic species similar to *C. perfringens*[a] | Spores | | Intestine, environment | Cellulitis, gas gangrene |
| *C. tetani* | Spores | Tetanospasmin | Environment | Tetanus |
| *C. botulinum* | Spores | Botulinum | Environment | Botulism |
| *C. dif cile* | Spores | A enterotoxin, B cytotoxin | Intestine, environment (nosocomial) | Antibiotic-associated diarrhea, enterocolitis |
| *Propionibacterium* | | | Skin | Rare opportunist |
| *Eubacterium* | | | Intestine | Rare opportunist |
| **Gram-negative bacilli** | | | | |
| *Bacteroides fragilis*[b] | Polysaccharide capsule | Enterotoxin | Intestine | Opportunist, abdominal abscess |
| *Bacteroides* species | | | Intestine | Opportunist |
| *Fusobacterium* | | | Mouth, intestine | Opportunist |
| *Prevotella* | Black pigment | | Mouth, urogenital | Opportunist |
| *Porphyromonas* | | | Mouth, urogenital | Opportunist |

[a] *C. histolyticum, C. noyyi, C. septicum,* and *C. sordellii.*

[b] The *Bacteroides fragilis* group includes *B. fragilis, B. distasonis, B. ovatus, B. vulgatus, B. thetaiotaomicron,* and six other species.

## Nonsporulating Gram-positive Bacilli

*Propionibacterium* is a genus of small pleomorphic bacilli sometimes called anaerobic diphtheroids because of their morphologic resemblance to corynebacteria. They are among the most common bacteria in the normal flora of the skin. *Eubacterium* is a genus that includes long slender bacilli commonly found in the colonic flora. These organisms are occasionally isolated from infections in combination with other anaerobes but rarely produce disease on their own.

Members of the normal flora

## Gram-negative Bacilli

Gram-negative, non–spore-forming bacilli are the most common bacteria isolated from anaerobic infections. In the past, most species were lumped into the genus *Bacteroides,* which still exists but now includes five other genera. Of these, *Fusobacterium, Porphyromonas,* and *Prevotella* are medically the most important. The *Bacteroides fragilis* group contains *B. fragilis* and 10 similar species noted for their virulence and production

Five genera are medically important

of $\beta$-lactamases. (Species outside this group generally lack these features and are more similar to the other anaerobic Gram-negative bacilli.) *B. fragilis* is a relatively short Gram-negative bacillus with rounded ends sometimes giving a coccobacillary appearance. The lipopolysaccharide (LPS) in its outer membrane has a much lower lipid content and thus lower toxic activity than that of most other Gram-negative bacteria. Virtually all *B. fragilis* strains have a polysaccharide capsule and are relatively oxygen tolerant through production of superoxide dismutase. *Prevotella, Porphyromonas,* and *Fusobacterium* are distinguished by biochemical and other taxonomic features. *Prevotella melaninogenica* forms a black pigment in culture, and *Fusobacterium,* as its name suggests, is typically elongated and has tapered ends.

*B. fragilis* group produces $\beta$-lactamase and superoxide dismutase

# ANAEROBIC INFECTIONS

## EPIDEMIOLOGY

Despite our constant immersion in air, anaerobes are able to colonize the many oxygen-deficient or oxygen-free microenvironments of the body. Often these are created by the presence of facultative organisms whose growth reduces oxygen and decreases the local oxidation-reduction potential. Such sites include the sebaceous glands of the skin, the gingival crevices of the gums, the lymphoid tissue of the throat, and the lumina of the intestinal and urogenital tracts. Except for infections with some environmental clostridia, anaerobic infections are almost always endogenous with the infective agent(s) derived from the patient's normal flora. The specific anaerobes involved are linked to their prevalence in the flora of the relevant sites as shown in Table 19–1. In addition to the presence of clostridia in the lower intestinal tract of humans and animals, their spores are widely distributed in the environment, particularly in soil exposed to animal excreta. The spores may contaminate any wound caused by a nonsterile object (eg, splinter, nail) or exposed directly to soil.

Low redox normal flora sites are the origin of most infections

Spore-forming clostridia also come from the environment

## PATHOGENESIS

The anaerobic flora normally live in a harmless commensal relationship with the host. However, when displaced from their niche on the mucosal surface into normally sterile tissues these organisms may cause life-threatening infections. This can occur as the result of trauma (eg, gunshot, surgery), disease (eg, diverticulosis), or isolated events (eg, aspiration). Host factors such as malignancy or impaired blood supply increase the probability that the dislodged flora eventually produce an infection. The organisms involved are anaerobes normally found at the mucosal site adjacent to the infection. For example, *B. fragilis,* which is one of the most common species in the colonic flora, is the organism most frequently isolated from intra-abdominal abscesses.

Anaerobes displaced from normal flora to deeper sites may cause disease

Trauma and host factors create the opportunity for infection

The relationship between normal flora and site of infection may be indirect. For example, aspiration pneumonia, lung abscess, and empyema typically involve anaerobes found in the oropharyngeal flora. The brain is not a particularly anaerobic environment, but brain abscess is most often caused by these same oropharyngeal anaerobes. This presumably occurs by extension across the cribriform plate to the temporal lobe, the typical location of brain abscess. In contaminated open wounds, clostridia can come from the intestinal flora or from spores surviving in the environment.

Flora may be aspirated or displaced at a distance

Brain abscess typically involves anaerobic bacteria

While gaining access to tissue sites provides the opportunity, additional virulence factors are needed for anaerobes to produce infection. Some anaerobic pathogens produce disease even when present as a minor part of the displaced resident flora, and other common members of the normal flora rarely cause disease. Classical virulence factors such as toxins and capsules are known only for the toxigenic clostridia and *B. fragilis,* but a feature such as the ability to survive brief exposures to oxygenated environments can also be viewed as a virulence factor. Anaerobes found in human infections are far more likely to produce catalase and superoxide dismutase than their more

Capsules and toxins are known for some anaerobes

Survival in oxidized conditions can be a virulence factor

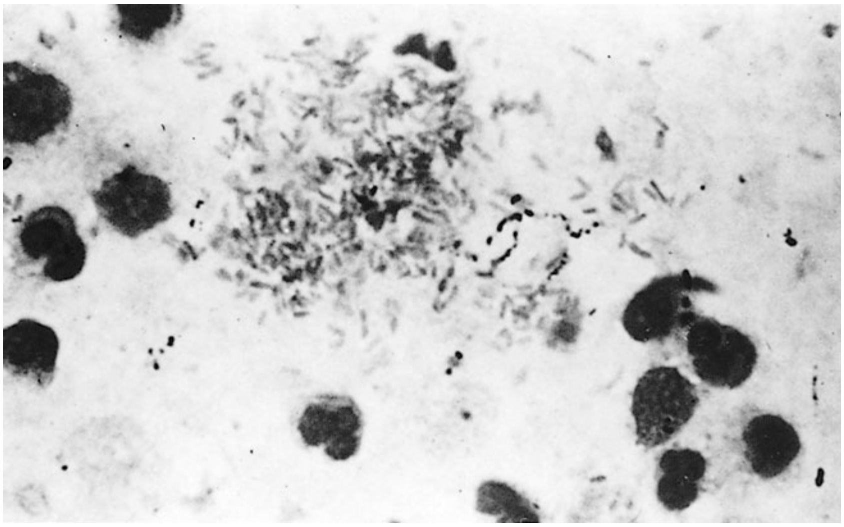

FIGURE 19–1
Gram smear of pus from an abdominal abscess showing polymorphonuclear leukocytes, large numbers of Gram-negative anaerobes and some peptostreptococci. (*Reproduced with permission of Schering Corporation, Kenilworth, NJ, the copyright owner. All rights reserved.*)

docile counterparts of the normal flora. Exquisitely oxygen-sensitive anaerobes are seldom involved, probably because they are injured by even the small amounts of oxygen dissolved in tissue fluids.

A related feature is the ability of the bacteria to create and control a reduced microenvironment, often with the apparent help of other bacteria. The great majority of anaerobic infections are mixed; that is, two or more anaerobes are present, often in combination with facultative bacteria such as *Escherichia coli* (Fig 19–1). In some cases the components of these mixtures are believed to synergize each other's growth either by providing growth factors or by lowering the oxidation-reduction potential. These conditions may have other advantages such as the inhibition of oxygen-dependent leukocyte bactericidal functions under the anaerobic conditions in the lesion. Anaerobes that produce specific toxins have a pathogenesis all their own, which will be discussed in the sections devoted to individual species.

Mixed infections may facilitate an anaerobic microenvironment

## ANAEROBIC INFECTIONS: CLINICAL ASPECTS

### MANIFESTATIONS

*Bacteroides, Fusobacterium,* and peptostreptococci, alone or together with other facultative or obligate anaerobes, are responsible for the overwhelming majority of localized abscesses within the cranium, thorax, peritoneum, liver, and female genital tract. As indicated earlier, the species involved relate to the pathogens present in the normal flora of the adjacent mucosal surface. Those derived from the oral flora also include dental infections and infections of human bites.

Abscesses are usually caused by *Bacteroides, Fusobacterium,* or peptostreptococci

In addition, anaerobes play causal roles in chronic sinusitis, chronic otitis media, aspiration pneumonia, bronchiectasis, cholecystitis, septic arthritis, osteomyelitis, decubitus ulcers, and soft tissue infections of patients with diabetes mellitus. Dissection of infection along fascial planes (necrotizing fasciitis) and thrombophlebitis are common complications. Foul-smelling pus and crepitation (gas in tissues) are signs associated with, but by no means exclusive to, anaerobic infections. As with other bacterial infections, they may spread beyond the local site and enter the bloodstream. The mortality rate of anaerobic bacteremias arising from nongenital sources is equivalent to the rates with bacteremias due to staphylococci or Enterobacteriaceae.

Foul-smelling pus suggests anaerobic infection

## DIAGNOSIS

Specimens must be direct and protected from oxygen

The key to detection of anaerobes is a high quality specimen, preferably pus or fluid taken directly from the infected site. The specimen needs to be taken quickly to the microbiology laboratory and protected from oxygen exposure while on the way. Special anaerobic transport tubes may be used or any air from the syringe in which the specimen was collected may be expressed. Actually, a generous collection of pus serves as an adequate transport medium unless transport is delayed for hours.

Gram staining is particularly useful

Anaerobic incubation jar provides atmosphere

Selective media inhibit facultative bacteria

A direct Gram-stained smear of clinical material demonstrating Gram-negative and/or Gram-positive bacteria of various morphologies is highly suggestive, often even diagnostic of anaerobic infection. Because of the typically slow and complicated nature of anaerobic culture, the Gram stain often provides the most useful information for clinical decision-making. Isolation of the bacteria requires the use of an anaerobic incubation atmosphere and special media protected from oxygen exposure. Although elaborate systems are available for this purpose, the simple anaerobic jar is sufficient for isolation of the clinically significant anaerobes. The use of media that contain reducing agents (cysteine, thioglycollate) and growth factors needed by some species further facilitates isolation of anaerobes. The polymicrobial nature of most anaerobic infections requires the use of selective media to protect the slow growing anaerobes from being overgrown by hardier bacteria, particularly members of the Enterobacteriaceae. Antibiotics, particularly aminoglycosides to which all anaerobes are resistant, are frequently used. Once the bacteria are isolated, identification procedures including morphology, biochemical characterization, and metabolic end-product detection by gas chromatography may begin.

## TREATMENT

Mixed infections and slow growth dictate empiric therapy

Abdominal infections require $\beta$-lactamase–resistant antimicrobics

As with most abscesses, drainage of the purulent material is the primary treatment, in association with appropriate chemotherapy. Antimicrobics alone may be ineffective because of failure to penetrate the site of infection. The selection of antimicrobics used is empiric to a degree; such infections typically involve mixed species, and cultural diagnosis is delayed by the slow growth and the time required to distinguish multiple species. In addition, antimicrobial susceptibility testing methods are slow and less standardized than they are for the rapidly growing bacteria. The usual approach involves selection of antimicrobics based on the expected susceptibility of the anaerobes known to produce infection at the site in question. For example, anaerobic organisms derived from the oral flora are often susceptible to penicillin, but infections below the diaphragm caused by fecal anaerobes such as *B. fragilis* are usually resistant to $\beta$-lactams. These latter infections are most likely to respond to clindamycin, metronidazole, or a cephalosporin such as cefoxitin, which is not inactivated by the $\beta$-lactamases produced by anaerobes.

# *CLOSTRIDIUM PERFRINGENS*

## BACTERIOLOGY

Double zone of hemolysis is characteristic

*C. perfringens* is a large, Gram-positive, nonmotile rod with square ends. It grows overnight on blood agar medium under anaerobic conditions, producing colonies surrounded by a double zone of hemolysis (Fig 19–2). In broth containing fermentable carbohydrate, growth of *C. perfringens* is accompanied by the production of large amounts of hydrogen and carbon dioxide gas, which can also be produced in necrotic tissues; hence the term gas gangrene.

*C. perfringens* produces multiple exotoxins that have different pathogenic significance in different animal species and serve as the basis for classification of the five types (A to E). Type A is by far the most important in humans and is found consistently in the

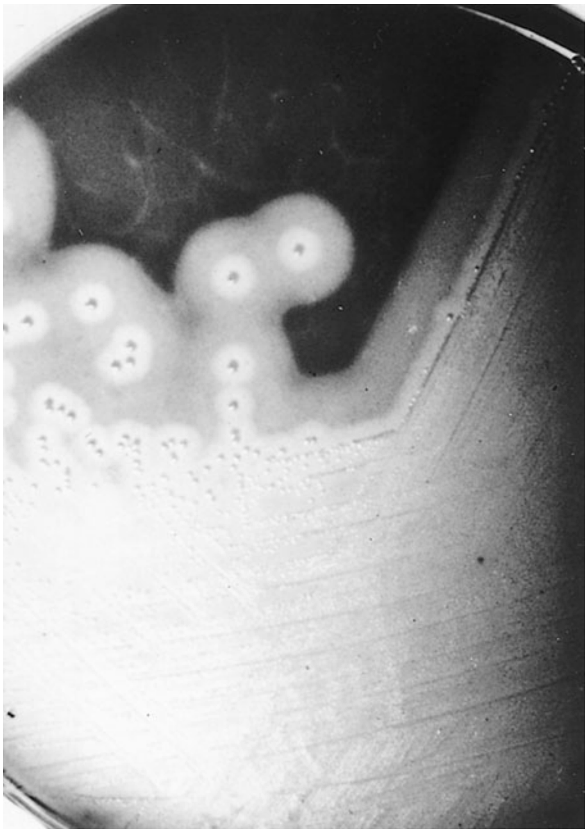

FIGURE 19–2
*C. perfringens* colonies on a blood agar plate showing double zone of hemolysis. The inner clear zone is caused by -toxin and the wider zone of incomplete hemolysis caused by -toxin.

colon and often in soil. The most important exotoxin is the ***α*-toxin**, a phospholipase that hydrolyzes lecithin and sphingomyelin, thus disrupting the cell membranes of various host cells, including erythrocytes, leukocytes, and muscle cells. The ***θ*-toxin** alters capillary permeability and is toxic to heart muscle. This pore-forming toxin is closely related to streptolysin O (see Chapter 17). When the **enterotoxin** is attached to enterocyte membranes, it causes an increase in intracellular calcium and altered membrane permeability which leads to loss of cellular uid and macromolecules.

Typing system is based on toxins

Phospholipase -toxin, pore-forming -toxin, and enterotoxin cause disease

## *C. perfringens* DISEASE

CLINICAL CAPSULE

*C. perfringens* produces a wide range of wound and soft tissue infections, many of which are no different from those caused by other opportunistic bacteria. The most dreaded of these, gas gangrene, begins as a wound infection but progresses to shock and death in a matter of hours. Another form of *C. perfringens*-caused disease, food poisoning, is characterized by diarrhea without fever or vomiting.

### EPIDEMIOLOGY

#### Gas Gangrene

Gas gangrene develops in traumatic wounds with muscle damage when they are contaminated with dirt, clothing, or other foreign material containing *C. perfringens* or another species of histotoxic clostridia. The clostridia can come from the patient's own intestinal ora or spores in the environment. Compound fractures, bullet wounds, or the kind of trauma seen in wartime are prototypes for this infection. A signi cant delay between the

Spores from the host or environment contaminate wounds

Delays allow multiplication

injury and definitive surgical management is an additional requirement. These conditions are more likely to occur in peacetime in a hiking accident in a remote area rather than in an automobile accident on a freeway.

### Clostridial Food Poisoning

*C. perfringens* can cause food poisoning if large numbers of an enterotoxin-producing strain are ingested. Outbreaks usually involve meat dishes such as stews, soups, or gravies. Clostridial food poisoning is one of the most common food-borne illnesses in developed countries.

## PATHOGENESIS

### Gas Gangrene

Low redox favors multiplication and toxin production

Toxins lead to shock

If the oxidation–reduction potential in a wound is sufficiently low, *C. perfringens* spores can germinate and can multiply, elaborating $\alpha$-toxin. The process passes along the muscle bundles, producing rapidly spreading edema and necrosis as well as conditions that are more favorable for growth of the bacteria. Very few leukocytes are present in the myonecrotic tissue. As the disease progresses, increased vascular permeability and systemic absorption of the toxin and inflammatory mediators leads to shock. $\theta$-toxin and oxygen deprivation due to the metabolic activities of *C. perfringens* are probable contributors. The basis for the profound systemic effects is not known, but toxin absorption seems probable, because fatal cases occur without bacteremia.

### Clostridial Food Poisoning

Spores survive cooking

Vegetative cells produce enterotoxin

The spores of some *C. perfringens* strains are often particularly heat resistant and can withstand temperatures of 100°C for an hour or more. Thus, spores that survive initial cooking can convert to the vegetative form and multiply if food is not refrigerated or is rewarmed. After ingestion, the enterotoxin is released into the upper gastrointestinal tract, causing a fluid outpouring in which the ileum is most severely involved.

# *C. perfringens*: CLINICAL ASPECTS

## MANIFESTATIONS

### Gas Gangrene

Wound pain evolves to edema and shock

Gas gangrene usually begins 1 to 4 days after the injury but may start within 10 hours. The earliest reported finding is severe pain at the site of the wound accompanied by a sense of heaviness or pressure. The disease then progresses rapidly with edema, tenderness, and pallor, which is followed by discoloration and hemorrhagic bullae. The gas is apparent as crepitance in the tissue, but this is a late sign. Systemic findings are those of shock with intravascular hemolysis, hypotension, and renal failure leading to coma and death. Patients are often remarkably alert until the terminal stages.

### Anaerobic Cellulitis

Gas is more likely than in gas gangrene

Anaerobic cellulitis is a clostridial infection of wounds and surrounding subcutaneous tissue in which there is marked gas formation (more than in gas gangrene) but in which the pain, swelling, and toxicity of gas gangrene are absent. This condition is much less serious than gas gangrene and can be controlled with less rigorous methods.

### Endometritis

Nonsterile abortion is greatest risk

If *C. perfringens* gains access to necrotic products of conception retained in the uterus, it may multiply and infect the endometrium. Necrosis of uterine tissue and septicemia with massive intravascular hemolysis due to $\alpha$-toxin may then follow. Clostridial uterine infection occurred more commonly in the past, usually after an incomplete illegal abortion with inadequately sterilized instruments.

### Food Poisoning

The incubation period of 8 to 24 hours is followed by nausea, abdominal pain, and diarrhea. There is no fever, and vomiting is rare. Spontaneous recovery usually occurs within 24 hours.

Diarrhea without fever or vomiting is most common

## DIAGNOSIS

Diagnosis is based ultimately on clinical observations. Bacteriologic studies are adjunctive. It is quite common, for example, to isolate *C. perfringens* from contaminated wounds of patients who have no evidence of clostridial disease. The organism can also be isolated from the postpartum uterine cervix of healthy women or from those with only mild fever. Occasionally, *C. perfringens* is even isolated from blood cultures of patients who do not develop serious clostridial infection. In clostridial food poisoning, isolation of more than $10^5$ *C. perfringens* per gram of ingested food in the absence of any other cause is usually sufficient to confirm the etiology of a characteristic food poisoning outbreak.

Isolation of clostridia alone is not sufficient

## TREATMENT AND PREVENTION

Treatment of gas gangrene and endometritis must be initiated immediately because these conditions are almost always fatal if untreated. Excision of all devitalized tissue is of paramount importance, because it denies the organism the anaerobic conditions required for further multiplication and toxin production. This often entails wide resection of muscle groups, hysterectomy, and even amputation of limbs. Administration of massive doses of penicillin is an important adjunctive procedure. Because nonclostridial anaerobes and members of the Enterobacteriaceae frequently contaminate injury sites, clindamycin and broad-spectrum cephalosporins are often added to the antibiotic regimen. Placement of patients in a hyperbaric oxygen chamber, which increases the tissue level of dissolved oxygen, has been shown to slow the spread of disease, probably by inhibiting bacterial growth and toxin production and by neutralizing the activity of $\theta$-toxin.

Surgical treatment is essential for gas gangrene and endometritis

Antibiotics and hyperbaric oxygen are useful

The most effective method for prevention of gas gangrene is the surgical debridement of traumatic injuries as soon as possible. Thorough cleansing, removal of dead tissue and foreign bodies, and drainage of hematoma limit organism multiplication and toxin production. Antimicrobic prophylaxis is indicated but cannot replace surgical debridement, because the antimicrobics may fail to reach the organism in devascularized tissues.

Debridement of dead tissue is best

Prevention involves good cooking hygiene and adequate refrigeration. There is growing evidence that enterotoxin-producing strains of *C. perfringens* may also be responsible for some cases of antimicrobic-induced diarrhea in a setting similar to *C. dif cile* .

# CLOSTRIDIUM TETANI

## BACTERIOLOGY

*C. tetani* is a slim, Gram-positive rod, which may stain Gram negative in very young or old cultures. It forms spores readily in nature and in culture, yielding a typical round terminal spore that gives the organism a drumstick appearance before the residual vegetative cell disintegrates. The organism is flagellate and motile. *C. tetani* requires strict anaerobic conditions. Its identity is suggested by cultural and biochemical characteristics, but definite identification depends on demonstrating its neurotoxic exotoxin. *C. tetani* spores remain viable in soil or culture for many years. It is resistant to most disinfectants and withstands boiling for several minutes.

Gram-positive rods decolorize readily

The most important product of *C. tetani* is its neurotoxic exotoxin, **tetanospasmin** or tetanus toxin, a metalloproteinase that enzymatically degrades a protein required for

Toxin blocks release of inhibitory neurotransmitters

Formaldehyde treatment removes toxicity but retains antigenicity

docking of neurotransmitter vesicles at the appropriate site on presynaptic membranes. Loss of this function prevents release of neurotransmitters used by inhibitory afferent motor neurons. The effect is unopposed firing of the motor neurons, generating spasms. The toxin is heat labile, antigenic, readily neutralized by antitoxin, and rapidly destroyed by intestinal proteases. Treatment with formaldehyde yields a nontoxic product or **toxoid** that retains the antigenicity of toxin and thus stimulates production of antitoxin.

# TETANUS

CLINICAL CAPSULE

The striking feature of tetanus is severe muscle spasms (or "lockjaw" when the jaw muscles are involved). This occurs despite minimal or no inflammation at the primary site of infection, which may be unnoticed even though the outcome is fatal. The disease is caused by in vivo production of a neurotoxin that acts centrally, not locally. Immunization with inactivated toxin, even after stepping on a rusty nail, prevents tetanus.

## EPIDEMIOLOGY

Spores from environment germinate in wounds

Nonsterile technique can lead to tetanus

The spores of *C. tetani* exist in many soils, especially those that have been treated with manure, and the organism is sometimes found in the lower intestinal tract of humans and animals. The spores are introduced into wounds contaminated with soil or foreign bodies. The wounds are often quite small, (eg, a puncture wound with a splinter). In many developing countries, the majority of tetanus cases occur in recently delivered infants when the umbilical cord is severed or bandaged in a nonsterile manner. Similarly, tetanus may follow an unskilled abortion, scarification rituals, female circumcision, and even surgery performed with nonsterile instruments or dressings.

## PATHOGENESIS

Trauma provides growth conditions

Tetanospasmin produced at the local site ascends through nerves to anterior horn

Blockage of reflex inhibition causes spasmodic contractions

The usual predisposing factor for tetanus is an area of very low oxidation–reduction potential in which tetanus spores can germinate, such as a large splinter, an area of necrosis from introduction of soil, or necrosis after injection of contaminated illicit drugs. Infection with facultative or other anaerobic organisms can contribute to the development of an appropriate anaerobic nidus for spore germination. Tetanus bacilli multiply locally and neither damage nor invade adjacent tissues. Tetanospasmin is elaborated at the site of infection and enters the presynaptic terminals of lower motor neurons, reaching the central nervous system (CNS) mainly by exploiting the retrograde axonal transport system in the nerves. In the spinal cord, it acts at the level of the anterior horn cells, where its blockage of postsynaptic inhibition of spinal motor reflexes produces spasmodic contractions of both protagonist and antagonist muscles. This process takes place initially in the area of the causative lesion but may extend up and down the spinal cord. Minor stimuli, such as a sound or a draft, can provoke generalized spasms.

# TETANUS: CLINICAL ASPECTS

## MANIFESTATIONS

The incubation period of the disease is from 4 days to several weeks. The shorter incubation period is usually associated with wounds in areas supplied by the cranial motor nerves, probably because of a shorter transmission route for the toxin to the CNS. In general, shorter incubation periods are associated with more severe disease.

FIGURE 19–3
Generalized tetanus. This child shows opisthotonic posturing caused by spasm of the spinal musculature. (*Photo courtesy of Anastacio de Queiroz Sousa, MD, Universidade Federal do Ceara, Fortaleza, Brazil, and Martin Cetron, MD, Centers for Disease Control and Prevention, Atlanta.*)

The diagnosis is clinical; neither culture nor toxin testing are useful. Although tetanus may be localized to muscles innervated by nerves in the region of the infection, it is usually more generalized. The masseter muscles are often the first to be affected, resulting in inability to open the mouth properly **(trismus);** this effect accounts for the use of the term **lockjaw** to describe the disease. As other muscles become affected, intermittent spasms can become generalized to include muscles of respiration and swallowing. In extreme cases, massive contractions of the back muscles (opisthotonos) develop (Fig 19–3).

Incubation period varies with distance to CNS

Masseter muscle contraction causes lockjaw

Untreated patients with tetanus retain consciousness and are aware of their plight, in which small stimuli can trigger massive contractions. In fatal cases, death results from exhaustion and respiratory failure. Untreated, the mortality caused by the generalized disease varies from 15 to more than 60%, according to the lesion, incubation period, and age of the patient. Mortality is highest in neonates and in elderly patients.

Respiratory failure leads to death

## TREATMENT

Specific treatment of tetanus involves neutralization of any unbound toxin with large doses of human tetanus immune globulin (HTIG), which is derived from the blood of volunteers hyperimmunized with toxoid. Most important in treatment are nonspecific supportive measures, including maintenance of a quiet dark environment, sedation, and provision of an adequate airway. Benzodiazepines are also used to indirectly antagonize the effects of the toxin. The value of antimicrobics is not clear. Because toxin binding is irreversible, recovery requires the generation of new axonal terminals.

Supportive treatment required until axons regenerate

## PREVENTION

Routine active immunization with tetanus toxoid, combined with diphtheria toxoid and pertussis vaccine (DTaP) for primary immunization in childhood and DT for adults, can completely prevent the disease. It has reduced the incidence of tetanus in the United States to less than 50 reported cases per year. Five doses of DT are recommended, to be given at the ages of 2, 4, 6, and 18 months, and once again between the ages of 4 and 6 years. Thereafter a booster of adult-type tetanus diphtheria toxoid should be given every 10 years. Unfortunately, routine childhood immunization is not administratively and economically feasible in many less well-developed countries, where as many as a million cases of tetanus occur annually. In such settings, immunization efforts have been focused on pregnant women, because transplacental transfer of antibodies to the fetus also prevents the highly lethal neonatal tetanus.

Childhood toxoid immunization prevents disease

Boosters required every 10 years

Unimmunized subjects with tetanus-prone wounds should be given passive immunity with a prophylactic dose of HTIG as soon as possible. This immunization provides immediate protection. Those who have had a full primary series of immunizations and appropriate boosters are given toxoid for tetanus-prone wounds if they have not been immunized within the previous 10 years in the case of clean minor wounds or 5 years for more contaminated wounds. If immunization is incomplete or the wound has been neglected and poses a serious

Passive immunization used when immunization is neglected risk of disease, HTIG is also appropriate. Penicillin therapy is a prophylactic adjunct in serious or neglected wounds but in no way alters the need for specific prophylaxis.

## *CLOSTRIDIUM BOTULINUM*

### BACTERIOLOGY

*C. botulinum* is a large Gram-positive rod much like the rest of the clostridia. Its spores resist boiling for long periods, and moist heat at 121°C is required for certain destruction. Germination of spores and growth of *C. botulinum* can occur in a variety of alkaline or neutral foodstuffs when conditions are sufficiently anaerobic.

Cells germinating from spores produce neurotoxin in food

Blockage of synaptic acetylcholine release causes paralysis

The major characteristic of medical importance is that when *C. botulinum* grows under these anaerobic conditions, it elaborates a family of neurotoxins of extraordinary toxicity. **Botulinum toxin** is the most potent toxin known in nature, with an estimated lethal dose for humans of less than 1 $\mu$g. Like tetanospasmin, botulinum toxin is a metalloproteinase that acts on the presynaptic membranes at neuromuscular junctions. Once bound, it cleaves proteins involved in the release of acetylcholine at the synapse. The major effect of this blockage of acetylcholine release is paralysis of the motor system, but it also causes dysfunction of the autonomic nervous system.

Toxin is destroyed by boiling

*C. botulinum* is classified into multiple types (A to G) based on the antigenic specificity of the neurotoxins. All of the toxins are heat labile and destroyed rapidly at 100°C but are resistant to the enzymes of the gastrointestinal tract. If unheated toxin is ingested, it is readily absorbed and distributed in the bloodstream.

### BOTULISM

CLINICAL CAPSULE

Botulism begins with cranial nerve palsies and develops into descending symmetrical motor paralysis, which may involve the respiratory muscles. No fever or other signs of infection occur. The time course depends on the amount of toxin present and whether it was ingested preformed in food or produced endogenously in the intestinal tract or a wound.

#### EPIDEMIOLOGY

Spores are widely distributed

Alkaline foods favor toxin production

Inadequately heated home-canned foods are most common source

Spores of *C. botulinum* are found in soil, pond, and lake sediments in many parts of the world, including the United States. If spores contaminate food, they may convert to the vegetative state, multiply, and produce toxin in storage under proper conditions. This may occur with no change in food taste, color, or odor. The alkaline conditions provided by vegetables, such as green beans, and mushrooms and fish support the growth of *C. botulinum,* and the acidic conditions provided by foods such as canned fruit do not support the growth of the bacterium. Botulism most often occurs after ingestion of home-canned products that have not been heated at temperatures sufficient to kill *C. botulinum* spores, although inadequately sterilized commercial fish products have also been implicated. Because the toxin is heat labile, food must be ingested uncooked or after insufficient cooking. Botulism often occurs in small family outbreaks in the case of home-prepared foods or less often as isolated cases connected to commercial products. Infant and wound botulism results when the toxin is produced endogenously, beginning with environmental spores that are either ingested or contaminate wounds.

## PATHOGENESIS

Food-borne botulism is an intoxication, not an infection. The ingested preformed toxin is absorbed in the intestinal tract and reaches its neuromuscular junction target via the bloodstream. Once bound there, its inhibition of acetylcholine release causes paralysis due to lack of neuromuscular transmission. The specific disease manifestations depend on the specific nerves to which the circulating toxin binds. Cardiac arrhythmias and blood pressure instability are believed to be due to effects of the toxin on the autonomic nervous system. As with tetanus, the damage to the synapse once the toxin has bound is permanent and recovery requires the sprouting of the presynaptic axons and formation of new synapses.

Preformed toxin is readily absorbed

Acetylcholine block leads to paralysis and autonomic effects

# BOTULISM: CLINICAL ASPECTS

## MANIFESTATIONS

Food-borne botulism usually starts 12 to 36 hours after ingestion of the toxin. The first signs are nausea, dry mouth, and, in some cases, diarrhea. Cranial nerve signs, including blurred vision, pupillary dilatation, and nystagmus, occur later. Symmetrical paralysis begins with the ocular, laryngeal, and respiratory muscles and spreads to the trunk and extremities. The most serious finding is complete respiratory paralysis. Mortality is 10 to 20%.

Blurred vision progresses to symmetrical paralysis

### Infant Botulism

A syndrome associated with *C. botulinum* that occurs in infants between the ages of 3 weeks and 8 months is now the most commonly diagnosed form of botulism. The organism is apparently introduced on weaning or with dietary supplements, especially honey, and multiplies in the infant's colon, with absorption of small amounts of toxin. The infant shows constipation, poor muscle tone, lethargy, and feeding problems and may have ophthalmic and other paralyses similar to those in adult botulism. Infant botulism may mimic sudden infant death syndrome. The benefits of antitoxin and antimicrobic agents have not been clearly established.

Nonsterile honey introduces spores to intestine

Lethargy, poor feeding occur in addition to adult signs

### Wound Botulism

Very rarely, wounds infected with other organisms may allow *C. botulinum* to grow. Wound botulism in parenteral users of cocaine and maxillary sinus botulism in intranasal users of cocaine has been reported. Disease similar to that from food poisoning may develop, or it may begin with weakness localized to the injured extremity. Botulism without an obvious food or wound source is occasionally reported in individuals beyond infancy. It is possible that some such cases result from ingestion of spores of *C. botulinum* with subsequent in vivo production of toxin in a manner similar to that in infant botulism.

Contaminated wounds of drug users are sites of toxin production

## DIAGNOSIS

The toxin can be demonstrated in blood, intestinal contents, or remaining food, but these tests require inoculation of mice and are performed only in reference laboratories. *C. botulinum* may also be isolated from stool or from foodstuffs suspected of responsibility for botulism.

Toxin can be detected in some laboratories

## TREATMENT AND PREVENTION

The availability of intensive supportive measures, particularly mechanical ventilation, is the single most important determinant of clinical outcome. With proper ventilatory support, mortality should be less then 10%. The administration of large doses of horse *C. botulinum* antitoxin is thought to be useful in neutralizing free toxin. Frequent hypersensitivity reactions related to the equine origin of this preparation makes it unsuitable for use in infants. Antimicrobial agents are given only to patients with wound botulism.

Supportive measures and antitoxin allow survival

Cooking food inactivates toxin

Adequate pressure cooking or autoclaving in the canning process kills spores, and heating food at 100°C for 10 minutes before eating destroys the toxin. Food from damaged cans or those that present evidence of positive inside pressure should not even be tasted because of the extreme toxicity of the *C. botulinum* toxin.

# *CLOSTRIDIUM DIFFICILE*

## BACTERIOLOGY

A and B toxins disrupt cytoskeleton signal transduction

A is a enterotoxin

B is a cytotoxin

*C. dif cile* is a Gram-positive rod that readily forms spores. Its early reputation for fastidious growth is responsible for its species epithet. Like the other clostridia described in this section, *C. dif cile* has a most important medical feature: its ability to produce toxins. In this species, two distinct large polypeptide toxins, A and B, with similar structure (45% homology) are released during late growth phases of the vegetative organism, perhaps at the time of cell lysis. Both toxins act in the cytoplasm by disrupting proteins involved in signal transduction, particularly those involving the actin cytoskeleton. The A toxin causes cell rounding and the disruption of intercellular tight junctions followed by altered membrane permeability and fluid secretion. The net effect is that of an enterotoxin, although inflammation and cytoxic activity are also present. The B toxin lacks the enterotoxic properties of the A toxin but has cytotoxic potency at least 10 times higher. The two toxins appear to act synergistically by a mechanism yet to be determined.

## *C. difficile* DIARRHEA

CLINICAL CAPSULE

*C. dif cile* is the most common cause of diarrhea that develops in association with the use of antimicrobial agents. The diarrhea ranges from a few days of intestinal fluid loss to life-threatening pseudomembranous colitis (PMC). This condition is associated with intense inflammation and the formation of a pseudomembrane composed of inflammatory debris on the mucosal surface.

### EPIDEMIOLOGY

Source is endogenous or environmental

*C. dif cile* is present in 2 to 5% of the general population, sometimes at higher rates among hospitalized persons and infants. More than two decades of the antibiotic era had elapsed before the medical importance of *C. dif cile* was recognized through its association with antibiotic-associated diarrhea (AAD). Although infection is endogenous in most cases, hospital outbreaks have clearly established that the environment can be the source as well.

Frequent cause of AAD

Major cause of PMC

*C. dif cile* is by no means the only cause of ADD, but it is the most common identifiable cause. In simple diarrhea following antimicrobial administration, this organism is responsible for approximately 30% of cases. As the disease progresses to colitis, the association is stronger, rising to 90% if PMC is present. Person-to-person transfer is very rare except in the instance of hospital-acquired *C. dif cile* infections, where environmental or hand contamination leads to infection of another patient.

### PATHOGENESIS

Antimicrobic effect on flora selects for *C. dif cile*

When *C. dif cile* becomes established in the colon of individuals with normal gut flora, few if any direct consequences result, probably because its numbers are dwarfed by the other flora. Alteration of the colonic flora with antimicrobics (particularly ampicillin,

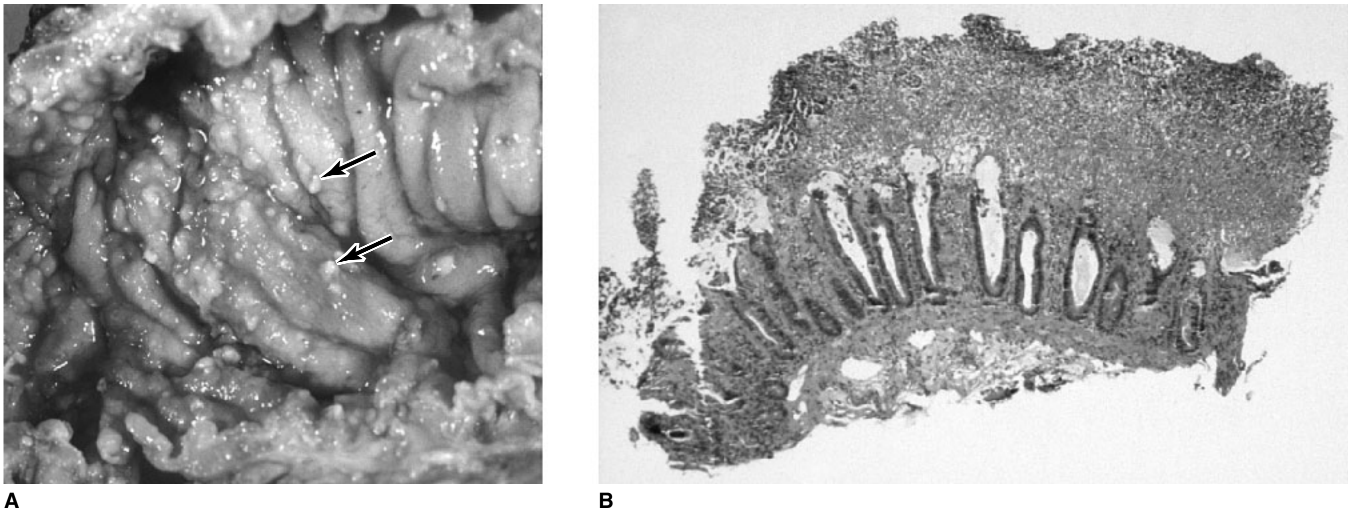


FIGURE 19-4
*Clostridium dif cile* pseudomembranous colitis. The plaques (arrows) on the surface of the intestinal mucosa (A) are composed of in ammatory cells and platelets (B).

cephalosporins, and clindamycin) favors *C. dif cile* in two ways. First, strains resistant to the antimicrobic can grow in its presence and assume a larger if not dominant position in the ora. Second, in an antimicrobial milieu, the readiness with which *C. dif cile* forms spores may favor its survival over non–spore-forming bacteria. In either case, the minor niche of the species is improved to the point at which the effect of its toxins on the colonic mucosa becomes signi cant.

Increased numbers make toxin more effective

Although the vast majority of strains produce both toxins, the enterotoxic properties of the A toxin seem to dominate in watery diarrhea cases. In PMC, the colonic mucosa is studded with in ammatory plaques, which may coalesce into an overlying "pseudomembrane" composed of brin, leukocytes, and necrotic colonic cells (Fig. 19–4). This picture ts better with the action of the c ytotoxic B toxin. It is intriguing that colonized newborn children, who lack the complex ora of adults, rarely suffer any clinical consequences even though toxin production can be demonstrated. The extent to which these differences are due to variability in toxin expression or intestinal receptors is unknown.

A enterotoxin stimulates watery diarrhea

B cytotoxin causes in ammation and pseudomembrane formation

## IMMUNITY

Antibody against the A toxin is associated with resolution of disease in experimental animals. This feature and the inverse relationship between severity of disease and anti-A antibody both support the importance of humoral immunity in *C. dif cile* diarrhea. Antibodies directed against the B toxin also appear to offer protection, but the relationship is less clear than with toxin A.

Antitoxin antibodies have protective effect

# *C. difficile* DIARRHEA: CLINICAL ASPECTS

## MANIFESTATIONS

Diarrhea is a frequent side effect of antimicrobic treatment. In *C. dif cile*–caused diarrhea, the onset is usually 5 to 10 days into the antibiotic treatment, but the range is from the rst day to weeks after cessation. The diarrhea may be mild and watery or bloody and accompanied by abdominal cramping, leukocytosis, and fever. In PMC, it progresses to a

Diarrhea ranges from mild to PMC

severe, occasionally lethal inflammation of the colon that can be demonstrated by endoscopic examination.

## DIAGNOSIS

Stool toxin detection is the primary diagnostic tool

Although selective media have been developed for isolation of *C. dif cile*, direct detection of toxins in the stool has largely replaced culture for diagnostic purposes. *C. dif cile* is the only pathogen for which detection of its toxin has become routine. The standard toxin assay requires demonstration and neutralization of cytopathic effect in cell culture. Newer enzyme immunoassays, which demonstrate toxin A and/or B in stool, are slightly less sensitive but less expensive and thus more widely available. False-positive results (toxins found but not associated with disease) may occur, particularly among infants.

## TREATMENT

Oral metronidazole or vancomycin reach bacteria in the intestine

Discontinuing the implicated antimicrobic usually results in the resolution of clinical symptoms. If patients are severely ill or fail to respond to drug withdrawal, they should receive metronidazole or vancomycin administered orally. The poor absorption of vancomycin is an advantage in this situation, but its use is now being restricted due to concern about its role in selecting resistant enterococci and other organisms. *C. dif cile* is susceptible to the penicillins and cephalosporins in vitro, but they are ineffective because of access in the intestinal lumen and the hazard of destruction by $\beta$-lactamases produced by other bacteria. Relapses or reinfections requiring retreatment occur in as many as 20% of patients.

# *BACTEROIDES FRAGILIS*

## BACTERIOLOGY

Oxygen-tolerant species that produce superoxide dismutase

Polysaccharide capsule is present

The *B. fragilis* group constitute the most common opportunistic pathogens of the genus *Bacteroides*. These slim, pale-staining, capsulate, Gram-negative rods form colonies overnight on blood agar medium. The implication of fragility in the name is misleading, because they are actually among the hardier and more easily grown anaerobes. Most strains produce superoxide dismutase and are relatively tolerant to atmospheric oxygen. *B. fragilis* has surface pili and a capsule composed of a polymer of two polysaccharides. The LPS endotoxin in the *B. fragilis* outer membrane is less toxic than that of most other Gram-negative bacteria, possibly due to modification or absence of the lipid A portion.

## *B. fragilis* DISEASE

CLINICAL CAPSULE

Deep pain and tenderness anywhere below the diaphragm is typical of the onset of *B. fragilis* infection. Depending on the extent and spread of the intra-abdominal abscess, fever and widespread findings of an acute abdomen may also be seen.

## EPIDEMIOLOGY

Endogenous infection mixed with other intestinal bacteria

Like the other Gram-negative anaerobes, *B. fragilis* infections are endogenous, originating in the patient's own intestinal flora. Although *B. fragilis* is among the most common of intestinal anaerobes, the frequent presence of this species in clinically significant infections is striking. It is typically mixed with other anaerobes and facultative bacteria. Human-to-human transmission is not known and seems unlikely.

## PATHOGENESIS

Pili and oxygen-tolerance aid initial stages

Capsule resists phagocytosis and stimulates abscess production

The relative oxygen tolerance of *B. fragilis* probably plays a role in its virulence by aiding its survival in oxygenated tissues in the period between its displacement from the intestinal flora and the establishment of a reduced local microenvironment. Its pili have adhesive properties, and the polysaccharide capsule confers resistance to phagocytosis and inhibits macrophage migration. The most distinguishing pathogenic feature of the organism is its ability to cause abscess formation. This capsule experimentally stimulates abscess formation, even in the absence of live bacteria. This property is not found in the capsular polysaccharides of organisms such as *Streptococcus pneumoniae*. *B. fragilis* and other *Bacteroides* species produce a number of extracellular enzymes (collagenase, fibrinolysin, heparinase, hyaluronidase) that may also contribute to the formation of the abscess.

Diarrheal enterotoxin is possible

Some strains of *B. fragilis* produce an enterotoxin that causes enteric disease in animals, and in some studies they have been associated a self-limited, watery diarrhea in children. Because these enterotoxin-producing strains are found in up to 10% of healthy individuals, their pathogenic importance is still undetermined.

## IMMUNITY

Cell-mediated immunity may be protective

Although it has been demonstrated that antibody to capsular polysaccharide facilitates classical complement pathway killing, there is no evidence that this confers immunity to reinfection. In contrast, there is some evidence that cell-mediated immunity may be protective.

# *B. fragilis*: CLINICAL ASPECTS

## MANIFESTATIONS

Some event that displaces *B. fragilis* along with other members of the intestinal flora is required to initiate infection; there is no evidence the organism is invasive on its own. This mucosal break may be the result of trauma or other disease states such as diverticulitis.

Abdominal pain and fever may evolve to peritonitis

Abscesses combined with anaerobes and Enterobacteriaceae

The local effects of the developing abscess include abdominal pain and tenderness, often with a low-grade fever. The subsequent course depends on whether the abscess remains localized or ruptures through to other sites such as the peritoneal cavity. This may cause several other abscesses or peritonitis. The course of illness is strongly influenced by the other bacteria in the abscess, particularly members of the Enterobacteriaceae. Spread to the bloodstream is more common with *B. fragilis* than any other anaerobe.

## TREATMENT

Cephalosporin resistant to $\beta$-lactamase is required

Drainage of abscesses and debridement of necrotic tissue are the mainstays of the treatment of *B. fragilis* infections, as with anaerobic infections in general. The accompanying antimicrobial therapy is complicated by the fact that abdominal *B. fragilis* isolates almost always produce a $\beta$-lactamase, which not only inactivates penicillin but other $\beta$-lactams, including many cephalosporins. Resistance to tetracycline is also common, but most strains are susceptible to chloramphenicol, clindamycin, and metronidazole. Among the $\beta$-lactams, cefoxitin and imipenem have been used effectively, as have combinations of a $\beta$-lactamase inhibitor (clavulanate, sulbactam) and a $\beta$-lactam (ampicillin, ticarcillin).

## ADDITIONAL READING


Hatheway CL. Toxigenic clostridia. *Clin Microbiol Rev* 1990;3:66–98. A comprehensive review of the historical aspects, organism characteristics, clinical diseases, and toxins of 13 species of clostridia.

Kasper DL, Onderdonk AB. Introduction: International symposium on anaerobic bacteria and bacterial infections. *Rev Infect Dis* 1990;12:S121–S252. This supplemental issue is devoted to the scientific papers given at an international symposium held in Monte Carlo. It is a comprehensive and timely presentation of the microbiologic and structural aspects, pathogenesis, immune mechanisms, susceptibility testing, and management of infections caused by the obligate anaerobes.

Midura TF. Update: Infant botulism. *Clin Microbiol Rev* 1996;9:119–125. This comprehensive review of this puzzling disease puts environmental aspects in perspective.

Murdoch DA. Gram positive anaerobic cocci. *Clin Microbiol Rev* 1998;11:81–120. This review contains more detail about the species of *Peptostreptococcus* than most students need, but the summaries of clinical syndromes are concise and informative.

Mylonakis E, Ryan ET, Calderwood SB. *Clostridium dif cile* –associated diarrhea. *Arch Intern Med* 2001;161:525–533. This well-illustrated review includes a complete discussion on the management of *C. dif cile* diarrhea.

Schreiner MS, Field E, Ruddy R. Infant botulism: A review of 12 years' experience at the Children's Hospital of Philadelphia. *Pediatrics* 1991;87:159–165. A well-referenced update of this disease.

Weber JT, Hibbs RG Jr, Darwish A, et al. A massive outbreak of type E botulism associated with traditional salted fish in Cairo. *J Infect Dis* 1993;167:451–454. A good example of how botulism can be spread widely.

CHAPTER 20

# *Neisseria*

KENNETH J. RYAN

*Neisseria* are Gram-negative diplococci. The genus contains two pathogenic and many commensal species, most of which are harmless inhabitants of the upper respiratory and alimentary tracts. The pathogenic species are *Neisseria meningitidis* (meningococcus), a major cause of meningitis and bacteremia, and *Neisseria gonorrhoeae* (gonococcus), the cause of gonorrhea.

## *NEISSERIA*: GENERAL FEATURES

*Neisseria* are Gram-negative cocci that typically appear in pairs with the opposing sides flattened, imparting a "kidney bean" appearance. They are nonmotile, non-spore forming, and non-acid fast. Their cell walls are typical of Gram-negative bacteria, with a peptidoglycan layer and an outer membrane containing endotoxic glycolipid complexed with protein. The structural elements of *N. meningitidis* and *N. gonorrhoeae* are the same, except that the meningococcus has a polysaccharide capsule external to the cell wall.

Gram-negative diplococci are bean-shaped

Gonococci and meningococci require an aerobic atmosphere with added carbon dioxide and enriched medium for optimal growth. Gonococci grow more slowly and are more fastidious than meningococci, which can grow on routine blood agar. All *Neisseria* are oxidase positive. Species are defined by growth characteristics and patterns of carbohydrate fermentation. Reagents are also available to distinguish *N. gonorrhoeae* and *N. meningitidis* from the other *Neisseria* by immunologic methods such as slide agglutination and immunofluorescence.

Gonococci are more fastidious than meningococci

All *Neisseria* are oxidase positive

Both pathogenic species possess pili, which vary in their antigenic composition, and several classes of outer membrane proteins (OMPs), which also vary antigenically. Various classes of the pili and OMPs of gonococci and meningococci have been separately named, but the structure and functional features of some are similar to each other and to diverse pathogens such as *Pseudomonas aeruginosa* and *Bacteroides* (Table 20–1). The outer membrane of pathogenic *Neisseria* contains a variant of lipopolysaccharide (LPS) in which the side chains are shorter and lack the repeating polysaccharide units found in the LPS of most other Gram-negative bacteria. This short chain neisserial LPS is called lipooligosaccharide (LOS). The lipid A and core oligosaccharide are structurally and functionally similar to other Gram-negative LPS. The pili, OMPs, and LOS are antigenic and have been used in typing schemes.

Pili and OMPs are present in both species

Outer membrane LOS has short side chains

TABLE 20–1

## Bacteriologic and Pathogenic Features of *Neisseria*

| | Growth | | Antigenic Structure | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Outer Membrane Proteins | | | | |
| Organism | Blood Agar | ML Agar[a] | Capsule | Pili | Adherence-Associated | Porins | Blocking Ab-Associated[b] | Transmission | Disease |
| *N. meningitidis* | + | + | Polysaccharide (12 serogroups[c]) | Class I,[d] II Antigenically diverse | Class 5 (4 variants) | PorA, PorB[e] | Class 4 | Inhalation of respiratory droplets | Meningitis, septic shock |
| *N. gonorrhoeae* | − | + | None[f] | Antigenically diverse[d] | Protein II or Opa (12 variants) | Protein I (A and B) | Protein III | Sexual contact of mucosal surfaces | Urethritis, cervicitis, PID |
| Other *Neisseria* species | + | − | None | Present | Unknown | Unknown | Absent | Normal respiratory flora | None |

*Abbreviations:* PID, pelvic inflammatory disease.

[a] Martin–Lewis or similar selective medium.

[b] Bind IgG in a way that interferes with bactericidal activity of antibodies directed at other antigens.

[c] A, B, C, H, I, K, L, X, Y, Z, 29E, W-135.

[d] Gonococcal and meningococcal class I are similar to each other and members of a class of bacterial pili with amino-terminal *N*-methylphenylalanine residues (*Bacteroides, Moraxella, Pseudomonas aeruginosa*).

[e] Two classes, similar to gonococcal protein I (A and B).

[f] LOS sialylation has some of the effects of a capsule (see text).

## *NEISSERIA MENINGITIDIS*

## BACTERIOLOGY

Meningococci produce medium-sized smooth colonies on blood agar plates after overnight incubation. Carbon dioxide enhances growth, but is not required. Twelve serogroups have been defined on the basis of the antigenic specificity of a polysaccharide capsule. The most important disease-producing serogroups are A, B, C, W-135, and Y. In addition to the group polysaccharides, individual *N. meningitidis* strains may contain two distinct classes of pili and multiple classes of OMPs. Some OMPs, porins, and adherence proteins have structural and functional similarities to those found in gonococci (see Table 20–1). The function of other OMPs is unknown.

Serogroups are based on the polysaccharide capsule

Some OMPs are similar to gonococci

## MENINGOCOCCAL DISEASE

CLINICAL CAPSULE

Meningococci are usually quiescent members of the nasopharyngeal flora but may produce fulminant infection of the bloodstream and/or central nervous system (CNS). There is little warning; localized infections that precede systemic spread are rarely recognized. The major disease is an acute, purulent meningitis with fever, headache, seizures, and mental signs secondary to inflammation and increased intracranial pressure. Even when the CNS is not involved, *N. meningitidis* infections have a marked tendency to be accompanied by rash, purpura, thrombocytopenia, and other manifestations associated with endotoxemia. This bacterium causes one of the few infections in which patients may progress from normal health to death in less than a day.

### EPIDEMIOLOGY

Meningococci are found in the nasopharyngeal flora of approximately 10% of healthy individuals. Transmission occurs by inhalation of aerosolized respiratory droplets. Close, prolonged contact such as occurs in families and closed populations promotes transmission. The estimated attack rate among family members residing with an index case is 1000 times higher than in the general population; this fact is evidence of the contagious nature of meningococcal infection. Other factors that foster transmission are contact with a virulent strain and susceptibility (lack of protective antibody). Typical settings of larger outbreaks are schools, dormitories, and camps for military recruits. In these close living circumstances, *N. meningitidis* spreads readily among newly exposed individuals, but disease develops only in those who lack group-specific antibody.

Nasopharyngeal carrier rate is 10%

Spread in is by respiratory droplets

The annual incidence of meningococcal infections in the United States varies between 0.5 and 1.5 cases per 100,000 population. Most cases are in children under 6 years of age. They occur as isolated cases, as sporadic small epidemics, or in small family or closed-population (school or day-care center) outbreaks. B, C, and Y are the most common serogroups involved. Group A strains are generally rare but historically have a more ominous epidemiologic potential. For unknown reasons, group A meningococci have the capability to cause widespread epidemics sweeping through communities, even countries. In the past these have appeared in 8- to 12-year cycles. It has been more than 40 years since group A strains have been responsible for significant disease in the United States, although epidemics have occurred in Brazil, China, the Sudan, Kenya, and South Africa.

B, C, and Y are the most common serogroups

Group A strains can cause widespread epidemics

## PATHOGENESIS

Meningococci range from carrier state to bacteremia

Pili attach to microvilli as prelude to invasion

The meningococcus is an exclusively human parasite; it can either exist as an apparently harmless member of the normal flora or produce acute disease. For most individuals, the carrier state is associated with acquisition of protective antibodies, but for some, spread from the nasopharynx to produce bacteremia, endotoxemia, and meningitis takes place too quickly for immunity to develop. Meningococci use pili for initial attachment to the microvilli of the nonciliated nasopharyngeal epithelium as a prelude to invasion. In the invasion process, the microvilli come in close contact with the bacteria, which then enter the cells in membrane-bound vesicles. Once inside meningococci quickly pass through the cytoplasm, exiting into the submucosa on the other side. In the process they damage the ciliated cells, possibly by direct release of endotoxin.

Proteins scavenge iron from transferrin

Polysaccharide capsules are antiphagocytic

LOS + sialic acid interferes with complement deposition

Once meningococci gain access to the submucosa, their ability to produce disease is enhanced by several factors that allow them to scavenge essential nutrients and evade the host immune response. One critical nutrient, iron, is supplied by *N. meningitidis* proteins, which are able to acquire it from the human iron transport protein transferrin. As with other encapsulated bacteria, the polysaccharide capsule enables meningococci to resist complement-mediated bactericidal activity and subsequent neutrophil phagocytosis. Meningococcal (and gonococcal) LPS/LOS also has features that facilitate evasion of host immune responses. Its chemical structure mimics sphingolipids found in the human brain enough for them to be recognized as self by the immune system. In addition, meningococci are able to incorporate sialic acid from host substrates as terminal substitutions of their LOS side chains. This sialyated LOS is able to downregulate complement deposition by binding serum factor H in a manner already described for streptococcal surface molecules such as group B streptococcal capsular sialic acid (see Chapter 17). The capsules of group B and C meningococci are also polymers of sialic acid.

Spread to blood and CNS produce systemic endotoxemia

LPS and peptidoglycan trigger cytokine release

Shedding outer membrane blebs hyperproduces LPS

The most serious manifestations of meningococcal disease are related to its spread to the bloodstream and, its namesake, the meninges. The exact mechanism of CNS invasion is unclear but is probably related to the level of the bacteremia. It occurs in the choroid plexus with its exceptionally high rate of blood flow. After CNS invasion, an intense subarachnoid space inflammatory response is generated, induced by the release of cell wall peptidoglycan fragments, LPS, and possibly other virulence factors causing the release of inflammatory cytokines. A prominent feature of meningococcal disease with or without CNS invasion is disseminated, potent, endotoxic activity (see Manifestations). When grown in culture, *N. meningitidis* readily releases endotoxin-containing blebs of its outer membrane from the cell surface as shown in Fig 20–1. It is not known whether this occurs in vivo, but the model of the meningococcus as a hyperproducer of LPS endotoxin certainly fits with its most serious disease manifestations.

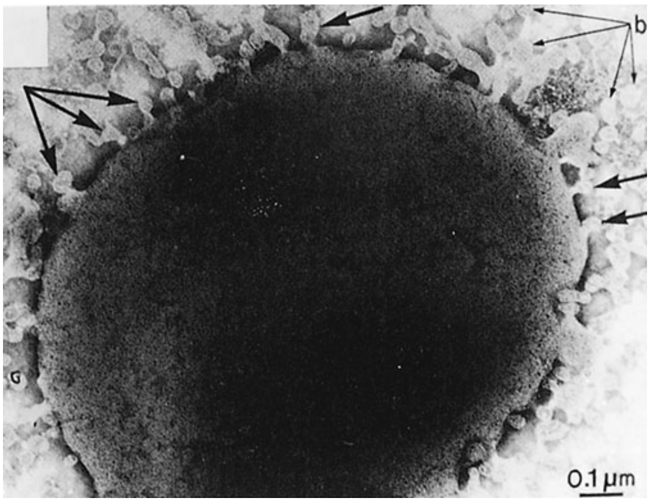


FIGURE 20–1
*Neisseria meningitidis*. Cell wall is shown shedding multiple "blebs" (*arrows*) containing lipopolysaccharide–endotoxin. Note the typical trilamellar Gram-negative cell wall structure in the wall and the blebs. (*Reprinted with permission from Devoe IW, Gilcrist JE.* J Exp Med *1973;138:1160, Figure 3.*)

## IMMUNITY

Immunity to meningococcal infections is related to group-specific antipolysaccharide antibody, which is bactericidal and facilitates phagocytosis. The bactericidal activity is due to complement-mediated cell lysis via the classical complement pathway. Individuals with deficiencies in the terminal complement components have an enhanced risk for meningococcal disease but not for other polysaccharide capsule pathogens such as *Haemophilus influenzae* type b (see Chapter 24).

Group-specific anticapsular antibody is protective

Complement component deficiencies enhance risk

The peak incidence of serious infection is between 6 months and 2 years of age. This corresponds to the nadir in the prevalence of antibody in the general population, which is the time between loss of transplacental antibody and the appearance of naturally acquired antibody (Fig 20–2). By adult life, serum antibody to one or more meningococcal serogroups is usually present but an immune deficit to the other serogroups remains. Infections appear when populations carrying virulent strains mix with susceptible individuals lacking group-specific antibody.

Most common age of infection is 6–24 months

Absence of antibody correlates with susceptibility

Protective antibody is stimulated by infection and through the carrier state, which produces immunity within a few weeks. Natural immunization may not require colonization with every serogroup or even with *N. meningitidis,* because antibody may be produced in response to cross-reactive polysaccharides possessed by other *Neisseria* or even other genera. For example, *Escherichia coli* strains of a particular serotype (K1) have a polysaccharide capsule identical to that of the group B meningococcus. These *E. coli* also have enhanced potential to produce meningitis in neonates.

Infection, carrier state, or other polysaccharides may stimulate antibody

Purified capsular polysaccharides are immunogenic, generating T cell–independent immune responses in which $IgG_2$ is the predominant antibody. As with other polysaccharide immunogens, these responses are not strong, particularly in early childhood when there is a relative deficiency of $IgG_2$. The group B polysaccharide differs from that of the other groups in failing to stimulate bactericidal antibody at all. This is believed to be due

T cell–independent mechanisms are involved

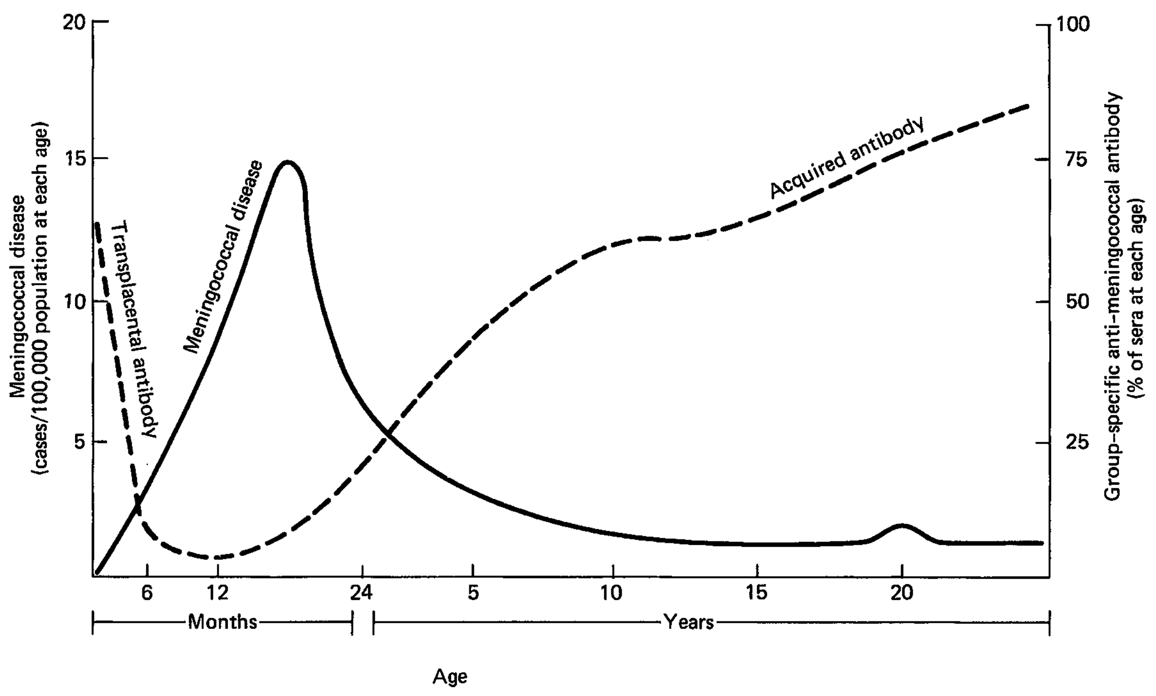


FIGURE 20–2
Immunity to the meningococcus. The inverse relationship between bactericidal meningococcal antibody and meningococcal disease is demonstrated. The "blip" in the disease curve around age 20 is attributable in part to military and other closed-population outbreaks. (*Adapted with permission from Goldschneider I, Gotschlich EC, Liu TY, Artenstein MS. Human immunity to the meningococcus I–V.* J Exp Med *1969;129:1307–1395.*)

Group B polysaccharide is not immunogenic

OMPs may be important in immunity

to the similarity of its sialic acid polymer to human brain antigens. That is, like the sialated LOS, it may be recognized as self by the immune system.

Exposed outer membrane proteins have also been shown to stimulate bactericidal antibody. Antibody directed against the porin PorA has demonstrated protection in animal models. PorA is present in the outer membrane of almost all meningococcal isolates but is subject to considerable antigenic variation.

# MENINGOCOCCAL DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

Meningitis is most frequent infection

Meningococcemia and rash may progress to DIC

Systemic features resemble endotoxic shock

The most frequent form of meningococcal infection is acute purulent meningitis, with clinical and laboratory features similar to those of meningitis from other causes (see Chapter 67). A prominent feature of meningococcal meningitis is the appearance of scattered skin petechiae, which may evolve into ecchymoses or a diffuse petechial rash. These cutaneous manifestations are signs of the disseminated intravascular coagulation (DIC) syndrome that is part of the endotoxic shock brought on by meningococcal bacteremia (meningococcemia). Meningococcemia sometimes occurs without meningitis and may progress to fulminant DIC and shock with bilateral hemorrhagic destruction of the adrenal glands (Waterhouse–Friderichsen syndrome). However, the disease is not always fulminant, and some patients have only low-grade fever, arthritis, and skin lesions that develop slowly over a period of days to weeks. Meningococci are a rare cause of other infections such as pneumonia, but it is striking that localized infections are almost never recognized in advance of systemic disease.

## DIAGNOSIS

Direct CSF Gram smears are diagnostic

Culture requires only blood agar

Direct Gram smears of cerebrospinal fluid (CSF) in meningitis usually demonstrate the typical bean-shaped, Gram-negative diplococci. Definitive diagnosis is by culture of CSF, blood, or skin lesions. Although *N. meningitidis* is reputed to be somewhat fragile, it requires no special handling for isolation from presumptively sterile sites such as blood and CSF. Growth is good on blood or chocolate agar after 18 hours of incubation. Speciation is based on carbohydrate degradation patterns or immunologic tests. Serogrouping may be performed by slide agglutination methods but has no immediate clinical importance.

## TREATMENT

Penicillin resistance is still rare

Penicillin is the treatment of choice for meningococcal infections because of its antimeningococcal activity and good CSF penetration. Resistance mediated by both $\beta$-lactamase and altered penicillin-binding proteins (PBPs) has been reported but is still extremely rare. Third-generation cephalosporins such as cefotaxime are effective alternatives to penicillin.

## PREVENTION

Rifampin is primary antimicrobic for chemoprophylaxis

Close contact with case is indication for prophylaxis

Until the development and spread of sulfonamide resistance in the 1960s, chemoprophylaxis with these agents was the primary means of preventing spread of meningococcal infections. Rifampin is now the primary chemoprophylactic agent, but ciprofloxacin has also been effective. Penicillin is not effective, probably because of inadequate penetration of the uninflamed nasopharyngeal mucosa. Selection of cases to receive prophylaxis is based on epidemiologic assessment. Risk is highest for siblings of the index case and declines with increasing age and less close contact. For example, an infant sibling sharing the same room as an affected individual would be at the highest risk. Typically, family members are given prophylaxis, but other adults are not. Common-sense exceptions, such as playmates and healthcare workers with very close contact (eg, mouth-to-mouth resuscitation), are made at the discretion of the physician. The presence or absence of nasopharyngeal carriage of *N. meningitidis* plays no role in this decision, because it does not accurately predict risk of disease.

Purified polysaccharide meningococcal vaccines have been shown to prevent group A and C disease in military and civilian populations, and a quadrivalent vaccine containing A, C, Y, and W-135 polysaccharides is now licensed for use in the United States. Meningococcal vaccines are currently used to control epidemics in populations at particular risk such as in military recruits and in those with unique predisposing factors such as complement deficiencies or asplenia. Routine immunization of children is not recommended.

A, C, Y, and W-135 polysaccharide vaccines are useful in high-risk populations

This reluctance for widespread use of meningococcal polysaccharide vaccines is ironic, because it was their development that led to the success of other vaccines made from capsular polysaccharides (see Additional Reading). Like other pure polysaccharides, these vaccines are ineffective in young children, because they stimulate immune responses that are underdeveloped in the first year of life (see Immunity).

Protein conjugate vaccines may enhance immunogenicity in children

With *H. influenzae* and now *Streptococcus pneumoniae,* this problem has been overcome by the development of polysaccharide–protein conjugate vaccines, which stimulate T cell–dependent responses (see Chapter 24). The protein conjugate approach, which is under investigation with *N. meningitidis,* faces a difficulty not shared by these other two pathogens—the failure of the group B polysaccharide to be immunogenic at all. If this is due to its similarity to human brain antigens, as suspected, it may not be overcome simply by protein conjugation. Group B causes one third of all disease, so no vaccine that omits it is likely to be completely successful. For this reason, other approaches such as the use of OMPs (eg, PorA) are being pursued. Genetically engineered vaccines based on the sequence of the entire group B meningococcal genome hold the promise of defining proteins that would immunize against all serogroups of *N. meningitidis.*

Nonimmunogenic serogroup B polysaccharide remains a problem

PorA and other OMPs are vaccine candidates

## NEISSERIA GONORRHOEAE

### BACTERIOLOGY

*N. gonorrhoeae* grows well only on chocolate agar and on specialized medium enriched to ensure its growth. It requires carbon dioxide supplementation. Small, smooth, nonpigmented colonies appear after 18 to 24 hours and are well developed (2 to 4 mm) after 48 hours. Gonococci possess numerous pili that extend through and beyond the outer membrane (Fig 20–3), which are structurally similar to those of meningococci (see Table 20–1). In general, only fresh virulent isolates have pili.

Chocolate agar and $CO_2$ are required

Fresh isolates have pili

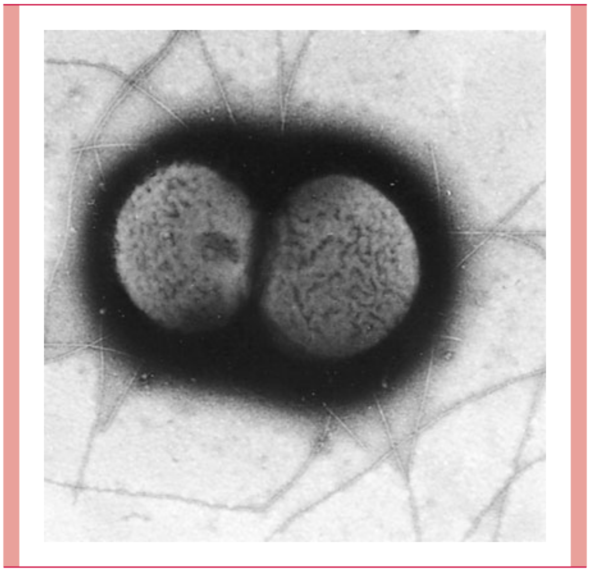

FIGURE 20–3
*Neisseria gonorrhoeae*. Surface pili are shown. These structures are associated with virulence and may mediate initial attachment to epithelial surfaces. (*Courtesy of Dr. John Swanson.*)

LPS, LOS, and OMPs are in outer membrane

Opa proteins are adherence OMPs

The gonococcal outer membrane is composed of phospholipids, LPS, LOS, and several distinct OMPs. The OMPs include porins (proteins IA and IB) and adherence proteins known as Opa or protein II. Opa proteins are a set of at least 12 proteins that get their name from the opaque appearance they give to colonies as a result of adhesion between gonococcal cells. A variable number of the Opa proteins may be expressed at any one time.

## ANTIGENIC VARIATION

Pili, OMPs, and LOS vary in gonococci and meningococci

*N. gonorrhoeae* and *N. meningitidis* are among several microorganisms whose surface structures are known to change antigenically from generation to generation during growth of a single strain. The mechanisms involved have been more extensively studied in gonococci but appear to be similar in both species. The antigenic structures of major interest are pili, Opa proteins, and LOS, for which there is evidence of antigenic variation both in vitro and in vivo. The genetic mechanisms involved are illustrated in Figure 20–4.

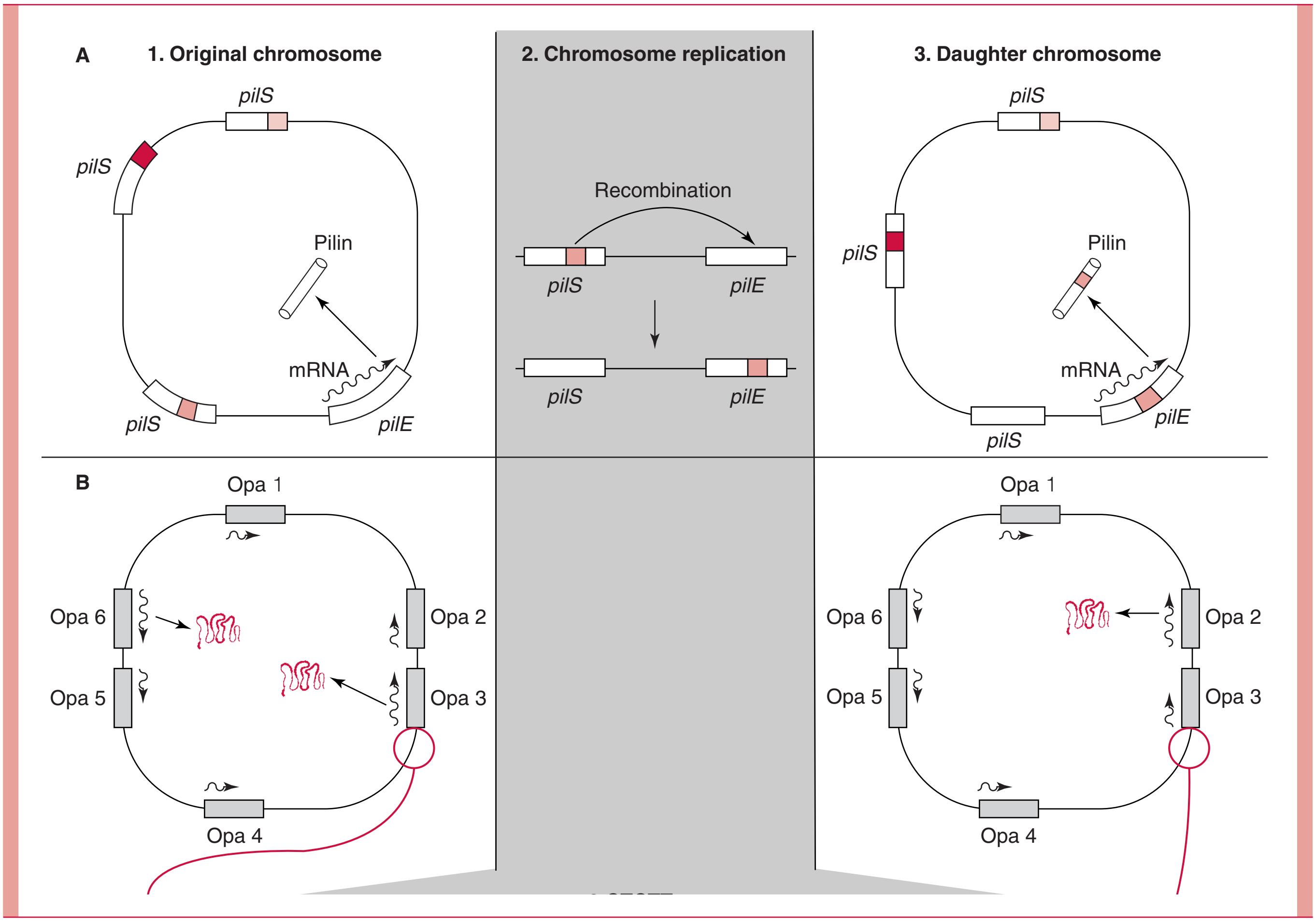


FIGURE 20–4

Antigenic variation of gonococci showing the mechanisms for change in the antigenic makeup of both pili and outer membrane Opa proteins. **A**. The chromosome contains multiple unlinked pilin genes, which are either expressing (*pilE*) or silent (*pilS*). The expressing gene is transcribing a mature pilin protein subunit. During chromosome replication, one of the *pilS* genes recombines with one of the *pilE* genes, donating some of its DNA. The new daughter chromosome now produces an antigenically different pilin based on transcription of the donated sequences into protein. **B.** The chromosome contains multiple Opa genes. Opa 3 and Opa 6 are "on" (producing protein), and the others are "off." During chromosome replication, replicative slippage in the leader peptide causes a five-base sequence (CTCTT) to be repeated variable numbers of times. Translation of the Opa will remain in-frame only if the number of added CTCTT nucleotides is evenly divisible by three. For the Opa gene in **B1**, the triplet code for alanine (GCA) is in-frame ($9 \times 5 = 45$. $45 \div 3 = 15$) but the **B3** it is out-of-frame.

Gonococcal pili are antigenically variable to an extraordinary extent. There are multiple genetic mechanisms, but the most important one appears to be recombinational exchange between the multiple pilin genes present in the chromosome of every strain. Some of these genes are complete and able to express pilin (*pilE*). Others are not due to lack of an effective promoter and are thus silent (*pilS*). When recombination between expression and silent loci results in the donation of new sequences to an expression locus, the result can be expression of a pilin with changes in its amino acid composition and thus its antigenicity. The recombination could also involve exogenous DNA from another cell or strain, because gonococci naturally take up species-specific DNA by transformation. The process is complex, involving other genes that play a role in the assembly of pili and their functional characteristics, such as cellular adhesion. The numerous possible outcomes include no pilin subunits, pilin subunits unable to assemble, mature pili with altered functional characteristics, and fully functional pili with a new antigenic makeup.

Genes for pilin subunits may be expressive or silent

Recombination between multiple genes occurs

Outcome may be nonfunctional or antigenically altered pili

The multiple gonococcal Opa proteins are each encoded by separate genes scattered around the genome. Various combinations of these genes may be either "on" or "off" at any one time. It has been observed that this switching between different Opa proteins occurs at a high rate per cell per generation. Control of the switch is via the number of repeats of a pentameric sequence (CTCTT) within the leader peptide encoding region of the gene. These are created by replicative slippage—a kind of "stuttering" during transcription. The number of repeats of this sequence varies widely (7 to 28), and when it comes time for translation the number of repeats determines whether the gene will be in or out of frame to translate the protein (Figure 20–4, B). Thus, by virtue of a translational frame shift mechanism, each Opa gene has its own switch, which can change with every cell cycle.

Multiple Opa genes may be "on" or "off"

Translational frame shift controls the switch

Variation in gonococcal LOS has been observed in volunteer subjects challenged with intraurethral *N. gonorrhoeae,* but the genetic mechanism is unknown. Taken together, these multifactorial, antigenic variations of the gonococcal surface may serve the dual purposes of escape from immune surveillance and timely provision of the ligands required to bind to human cell receptors.

LOS also varies antigenically

# GONORRHEA

CLINICAL CAPSULE

In contrast to meningococcal disease, gonorrhea is primarily localized to mucosal surfaces with relatively infrequent spread to the bloodstream or deep tissues. Infection is sexually acquired by direct genital contact, and the primary manifestation is pain and purulent discharge at the infected site. In men, this is typically the urethra, and in women, the uterine cervix. Direct extension of the infection up the fallopian tubes produces fever and lower abdominal pain, a syndrome called pelvic inflammatory disease (PID). For women, sterility or ectopic pregnancy can be long-term consequences of gonorrhea.

## EPIDEMIOLOGY

Although official reports of gonorrhea in the United States, which represent approximately 50% of the true cases, have been declining for 20 years, the disease is still one of our greatest public health problems. The overall incidence is now 130 cases per 100,000 population, but the rates for adolescents are alarmingly high and increasing by 10% a year. The highest rates are in women between the ages of 15 and 19 years (761/100,000) and men between the ages of 20 and 24 years (564/100,000). No truly effective means of control is yet in sight. Our ability to stem the tide of changed sexual mores continues to be hampered by lack of an effective means to detect asymptomatic cases, resistance of *N. gonorrhoeae* to antibiotics (see Treatment), and, to some extent, lack of appreciation of the importance of this disease. The latter is evidenced by failure of patients to seek

Rates among adolescents are very high and increasing

Inability to detect asymptomatic cases hampers control medical care and of physicians to report cases to public health authorities in order to protect the privacy of their patients. In the minds of too many, syphilis is dreaded and "unclean," whereas gonorrhea is only "the clap" ("clap" is from the archaic French clapoir, "a rabbit warren"; later, "a brothel").

The major reservoir for continued spread of gonorrhea is the asymptomatic patient. Screening programs and case contact studies have shown that almost 50% of infected women are asymptomatic or at least do not have symptoms usually associated with venereal infection. Most men (95%) have acute symptoms with infection. Many who are not treated become asymptomatic but remain infectious. Asymptomatic male and female patients can remain infectious for months. The attack rates for those engaging in genital intercourse with an infected patient are estimated to be 20 to 50%. The organism may also be transmitted by oral–genital contact or by rectal intercourse. When all of these factors operate in a sexually active population, it is easy to explain the high prevalence of gonorrhea. Although gonococci can survive for brief periods on toilet seats, nonsexual transmission is extremely rare. Fomite transmission of a purulent vulvovaginitis in prepubescent girls has been reported, but virtually all gonococci isolated from children can be traced to sexual abuse by an infected adult.

Risk of sexual contact is up to 50%

Asymptomatic cases are highest in women

Nonsexual transmission is rare

## PATHOGENESIS

### Attachment and Invasion

Gonococci are not normal inhabitants of the respiratory or genital flora. When introduced onto a mucosal surface by sexual contact with an infected individual, adherence ligands such as pili, Opa proteins, and possibly LOS allow initial attachment of the bacteria to receptors (CD46, CD66) on nonciliated epithelial cells. Pili are the primary mediators of adherence to urethral and vaginal epithelium, nonciliated fallopian tube cells, sperm, and neutrophils. Opa proteins are involved in cervical and urethral epithelial cell adherence and in adhesion between gonococcal cells.

Pili and Opa proteins mediate attachment to nonciliated epithelium

Following attachment, gonococci invade epithelial cells. The microvilli surround the bacteria and appear to draw them into the host cell in the same manner as meningococci. This process is called **parasite-directed endocytosis** because it appears to be initiated by bacterial rather than host cell factors and involves cells which are not ordinarily phagocytic. Gonococcal OMPs such as protein IA and some of the Opa proteins appear to facilitate this process. Once inside, the bacteria transcytose the cell and exit through the basal membrane to enter the submucosa.

Invasion initiated by protein IA and Opa proteins

Bacteria pass to submucosa

### Survival in the Submucosa

Once in the submucosa, the bacteria must survive and resist innate host defenses as well as defenses that may have been acquired from previous infection. As with meningococci, receptors on the gonococcal surface enable the organisms to scavenge iron needed for growth from the human iron transport proteins transferrin and lactoferrin. Although gonococci lack the polysaccharide capsule of the meningococcus, they still have multiple mechanisms that protect them against serum complement and antibody. One of these, LOS sialyation, appears to provide a mechanism for blocking C3b deposition that is identical to that of the encapsulated bacteria. In a sense, the gonococci create their own "capsule" by incorporating host sialic acid into their LOS. Another mechanism for phenotypic serum resistance is the binding of antibodies to another class of OMPs found in both gonococci and meningococci (see Table 20–1). IgG bound to these OMPs appears to block the bactericidal activity of antibodies directed against other surface antigens such as protein I. Blocking antibodies have been found in patients with repeated gonococcal infection.

Receptors scavenge iron

Sialated LOS acts like a capsule

Some antibodies to OMPs have blocking effect on bactericidal activity

Even when phagocytes do encounter gonococci, surface factors such as pili and Opa proteins interfere with effective phagocytosis. The organisms are also able to defend against oxidative killing inside the phagocyte by upregulation of catalase production. Taken together, these factors provide ample evidence that killing by neutrophils is

Phagocytosed gonococci resist killing

sufficiently retarded to allow prolonged survival of gonococci in mucosal and submucosal locations.

## Spread and Dissemination

In contrast to meningococci, *N. gonorrhoeae* bacteria tend to remain localized to genital structures, causing inflammation and local injury, which no doubt facilitate their continued venereal transmission. Purulent exudates containing "sticky" clusters of gonococci held together by Opa proteins could be the primary infectious unit. Infection may spread to deeper structures by progressive extension to adjacent mucosal and glandular epithelial cells. These include the prostate and epididymis in men and the paracervical glands and the fallopian tubes in women. Spread to the fallopian tubes may be facilitated by pilus-mediated attachment to sperm and then to the microvilli of nonciliated fallopian tube cells. Injury to the fallopian epithelium seems to be mediated by LPS/LOS and fragments of gonococcal cell wall peptidoglycan. Gonococci are known to turn over their peptidoglycan rapidly during exponential growth, releasing peptidoglycan fragments into the local environment. Injury by this mechanism has been demonstrated in fallopian tube organ cultures and presumably may also operate at other sites.

Disease remains localized

Local spread is to epididymis and fallopian tubes

Peptidoglycan shedding causes local injury

In a small proportion of infection, organisms reach the bloodstream to produce disseminated gonococcal infection (DGI). When this happens, the systemic findings have their own pattern (see Manifestations) and seldom take on the endotoxic shock picture of meningococcemia. Although differences have been noted between *N. gonorrhoeae* strains that remain localized and those that produce DGI, their connection to pathogenesis is unknown. Both DGI and salpingitis tend to begin during or shortly after completion of menses. This may relate to changes in the cervical mucus and reflux into the fallopian tubes during menses.

DGI differs from meningococcal endotoxic shock

Reflux during menses may facilitate spread

## Genetic Regulation of Virulence

Through all the stages of gonorrhea, gonococci are able to use a particularly rich variety of genetic mechanisms in deployment of the virulence factors described above at the right time. Some are regulatory responses to environmental cues, such as iron in relation to iron-binding proteins, while others involve the changes in the genome. Antigenic changes in both pili and Opa proteins have been demonstrated in human infection, including the isolation of antigenic variants from different sites in the same patient. These presumably take place by the recombinational and translational mechanisms (see Antigenic Variation) as the organisms replicate in the patient.

Regulation, recombination, and translational changes deploy virulence factors

# IMMUNITY

The apparent lack of immunity to gonococcal infection has long been a mystery. Among sexually active persons with multiple partners, repeated infections are the rule rather than the exception. Both serum and secretory antibodies are generated during natural infection but the levels are generally low, even after repeated infections. Another aspect is that even when antibodies are formed, antigenic variation defeats their effectiveness and allows the gonococcus to escape immune surveillance. Antigenic variation of pili, Opa proteins, and LOS is particularly likely to be important. Outbreaks have been traced to a single strain that demonstrated multiple pilin variations and Opa types in repeated isolates from the same individual or from sexual partners. In experimental models, passive administration of antibody directed against one pilin type has been followed by emergence of new pilin variants. Changes in Opa proteins may also occur, as suggested by differences in its expression in mucosal versus tubal isolates. It appears that although some immunity to gonococcal infection is present, its effectiveness is compromised by the ability of the organism to change key structures during the course of infection.

Antibody response is weak

Gonococcus varies multiple structures to avoid immune surveillance

# GONORRHEA: CLINICAL ASPECTS

## MANIFESTATIONS

### Genital Gonorrhea

Urethritis and endocervicitis are primary infections

In men, the primary site of infection is the urethra. Symptoms begin 2 to 7 days after infection and consist primarily of purulent urethral discharge and dysuria. Although uncommon, local extension can lead to epididymitis or prostatitis. The endocervix is the primary site in women, in whom symptoms include increased vaginal discharge, urinary frequency, dysuria, abdominal pain, and menstrual abnormalities. As mentioned previously, symptoms may be mild or absent in either sex, particularly women.

### Other Local Infections

Rectal and pharyngeal infections relate to sexual practices

Rectal gonorrhea occurs after rectal intercourse or, in women, after contamination with infected vaginal secretions. This condition is generally asymptomatic but may cause tenesmus, discharge, and rectal bleeding. Pharyngeal gonorrhea is transmitted by oral–genital sex and, again, is usually asymptomatic. Sore throat and cervical adenitis may occur. Infection of other structures near primary infection sites, such as Bartholin's glands in women, may lead to abscess formation.

Transmission at birth causes ophthalmia neonatorum

Inoculation of gonococci into the conjunctiva produces a severe, acute, purulent conjunctivitis. Although this infection may occur at any age, the most serious form is gonococcal ophthalmia neonatorum, a disease acquired by a newborn from an infected mother. The disease was formerly a common cause of blindness, which is now prevented by the use of prophylactic topical eye drops or ointment (silver nitrate, erythromycin, or tetracycline) at birth.

### Pelvic Inflammatory Disease (PID)

Salpingitis and pelvic peritonitis cause scaring and infertility

The clinical syndrome of PID develops in 10% to 20% of women with gonorrhea. The findings include fever, lower abdominal pain (usually bilateral), adnexal tenderness, and leukocytosis with or without signs of local infection. These features are caused by spread of organisms along the fallopian tubes to produce salpingitis and into the pelvic cavity to produce pelvic peritonitis and abscesses. PID is also known to develop when other genital pathogens ascend by the same route. These organisms include anaerobes and *Chlamydia trachomatis,* which may appear alone or mixed with gonococci. The most serious complications of PID are infertility and ectopic pregnancy secondary to scarring of the fallopian tubes.

### Disseminated Gonococcal Infection (DGI)

Skin rash, arthralgia, and arthritis are associated with bacteremia

Purulent arthritis involves large joints

Any of the local forms of gonorrhea or their extensions such as PID may lead to bacteremia. In the bacteremic phase, the primary features are fever; migratory polyarthralgia; and a petechial, maculopapular, or pustular rash. Some of these features may be immunologically mediated; gonococci are infrequently isolated from the skin or joints at this stage despite their presence in the blood. The bacteremia may lead to metastatic infections such as endocarditis and meningitis, but the most common is purulent arthritis. The arthritis typically follows the bacteremia and involves large joints such as elbows and knees. Gonococci are readily cultured from the pus.

## DIAGNOSIS

### Gram Smear

Direct smear is useful in men

The presence of multiple pairs of bean-shaped, Gram-negative diplococci within a neutrophil is highly characteristic of gonorrhea when the smear is from a genital site (Fig 20–5). The direct Gram smear is more than 95% sensitive and specific in symptomatic men.

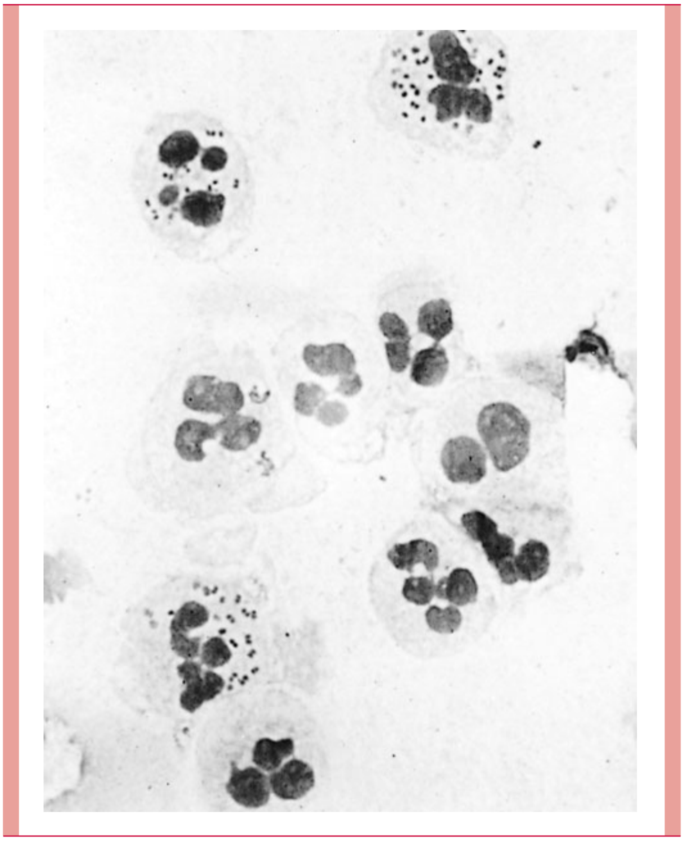

FIGURE 20–5
Gram smear of urethral exudate of an acute case of gonorrhea in a male. Note typical intracellular diplococci in polymorphonuclear leukocytes and the Gram-negative gonococci.

Unfortunately, it is only 50 to 70% sensitive in women, and its specificity is complicated by the presence of other bacteria in the female genital flora that may have a similar morphology. Experience is required in reading smears, particularly in women. Although a positive Gram smear is generally accepted as diagnostic in men, it should not be used as the sole source for diagnosis when the findings are unexpected or have social (divorce) or legal (rape, child abuse) implications.

Interfering flora complicates interpretation in women

## Culture

Attention to detail is necessary for isolation of the gonococcus, because it is a fragile organism that is often mixed with hardier members of the normal flora. Success requires proper selection of culture sites, protection of specimens from environmental exposure, culture on appropriate media, and definitive laboratory identification. In men, the best specimen is urethral exudate or urethral scrapings (obtained with a loop or special swab). In women, cervical swabs are preferred over urethral or vaginal specimens. The highest diagnostic yield in women is with the combination of a cervical and an anal canal culture, because some patients with rectal gonorrhea have negative cervical cultures. Throat or rectal cultures in men are needed only if indicated by the patient's sexual practices.

Urethra and cervix are preferred culture sites

Swabs may be streaked directly onto culture medium or transmitted to the laboratory in a suitable transport medium if the delay is not more than 4 hours. Laboratory requests must specify the suspicion of gonorrhea, so that media that satisfy the nutritional requirements of the gonococcus and inhibit competing normal flora can be seeded. The most common medium is Martin–Lewis agar, an enriched selective chocolate agar. The exact formulation has changed over the years, but includes antimicrobics active against Gram-positive bacteria (vancomycin), Gram-negative bacteria (colistin, trimethoprim), and fungi (nystatin, anisomycin) at concentrations that do not inhibit *N. gonorrhoeae*.

Transport media required unless plating is immediate

Selective medium inhibits competing flora

Colonies appear after 1 to 2 days of incubation in carbon dioxide at 35°C. They may be identified as *Neisseria* by demonstration of typical Gram stain morphology and a positive oxidase test. Classically, speciation is by carbohydrate degradation pattern, but this

Isolates are identified by fermentation or immunoassay approach has been replaced by immunologic procedures (immunofluorescence, coagglutination, enzyme immunoassay) using monoclonal antibodies to unique antigens such as protein I. *Neisseria* species other than *N. gonorrhoeae* are unusual in genital specimens, but speciation is the only way to be certain of the diagnosis.

### Direct Detection

DNA amplification methods are sensitive but expensive

Much effort has been directed at developing immunoassay and nucleic acid hybridization methods that detect gonococci in clinical specimens without culture. Such methods could have particular importance for screening populations where culture is impractical. Of these only the DNA amplification methods have the sensitivity to substitute for culture. The main barrier to their broader use is cost, which may be overcome by combining them with *Chlamydia* detection that targets the same clinical population.

### Serology

No serologic test

Attempts to develop a serologic test for gonorrhea have not yet achieved the needed sensitivity and specificity. A test that would detect the disease in asymptomatic patients would be very useful in control of this disease.

## TREATMENT

Compliance dictates treatment on first encounter

The treatment of gonorrhea, as with other sexually transmitted diseases, includes individual patient issues as well as public health concerns. Patients who do not complete a course of treatment once they begin to feel better present a risk of continued transmission and selection of resistant strains. For this reason, definitive treatment at the time of the initial visit has been the favored approach. For decades, this was easily accomplished with a single intramuscular injection of penicillin G.

PBP alterations cause incremental resistance

$\beta$-Lactamase–producing strains are highly resistant

Penicillin is no longer used, because of the development of two mechanisms of resistance. The first to be recognized was a slightly decreased susceptibility linked to altered PBPs. Over three decades, the minimum inhibitory concentrations (MICs) of altered PBP gonococci gradually increased (0.1 to more than 4.0 $\mu$g/mL), along with the dosage of the single injection favored for outpatient treatment. Eventually, the volume required to deliver the recommended dose began to exceed that which could be humanely administered, even injecting both buttocks. A second resistance mechanism, penicillinase production, first appeared in the Far East during the Viet Nam war and by the mid-1980s was endemic throughout the world. These strains produce a plasmid-encoded $\beta$-lactamase identical to that of members of the Enterobacteriaceae and have MICs that far exceed achievable therapeutic levels.

Ceftriaxone, quinolones, and azithromycin are recommended therapy

Quinolone and azithromycin resistance is still uncommon

This situation has caused a shift in treatment of genital gonorrhea to third-generation cephalosporins, because of their resistance to the $\beta$-lactamases prevalent in gonococci. The recommended agents have high enough activity to still be used as single dose treatment either intramuscularly (ceftriaxone) or orally (cefixime). Other agents recommended for primary treatment include fluoroquinolones (ciprofloxacin or ofloxacin) and azithromycin. Doxycycline is also effective but must be given orally for 7 days. Doxycycline and azithromycin have the additional advantage of also being effective against *Chlamydia trachomatis* (see Chapter 30), which may also be present in up to one third of gonorrhea cases. Resistance to quinolones is frequent enough to limit their use in some parts of the world. Azithromycin resistance is just beginning to be reported.

## PREVENTION

Condoms should block transmission

Vaccine strategies await better understanding of immunity

Methods to block direct mucosal contact (condoms) or inhibit the gonococcus (vaginal foams, douches) have been shown to provide protection against gonorrhea if used properly. The classic public health methods of case–contact tracing and treatment are important but difficult due to the size of the infected population. The availability of a good serologic test would greatly aid control, as it has for syphilis. The development of a gonococcal vaccine awaits further understanding of immunity and its relationship to the shifting target provided by the gonococcus.

## ADDITIONAL READING

Goldschneider I, Gotschlich EC, Liu TY, Artenstein MS. Human immunity to the meningococcus I–V. *J Exp Med* 1969;129:1307–1395. This series of five classic papers from the Walter Reed Army Institute of Research define the basis of immunity to *Neisseria meningitidis* and lay out the steps which lead to the development of vaccines from the polysaccharide capsule.

Pizza M, Rappuoli R, et al [37 authors]. Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. *Science* 2000;287:1816–1820. This progress report is by an Italian group that is using an entirely genetic approach to development of meningococcal vaccines. The researchers derive their candidate proteins from the chromosome sequence—not the organism itself.

Van Deuren M, Brandtzaeg, Van der Meer, JMM. Update on meningococcal disease with emphasis on pathogenesis and clinical management. *Clin Microbiol Rev* 2000;13:144–166. This review presents a detailed but clear discussion of how the virulence factors of the meningococcus are translated into septic shock in the infected patient.

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CHAPTER 21

# Enterobacteriaceae

KENNETH J. RYAN

The Enterobacteriaceae are a large and diverse family of Gram-negative rods, members of which are both free-living and part of the indigenous flora of humans and animals. A few are adapted strictly to living in humans. The Enterobacteriaceae grow rapidly under aerobic or anaerobic conditions and are metabolically active. They are by far the most common cause of **urinary tract infections (UTIs),** and a limited number of species are also important etiologic agents of **diarrhea.** Spread to the bloodstream causes Gram-negative endotoxic shock, a dreaded and often fatal complication.

## GENERAL CHARACTERISTICS

## BACTERIOLOGY

### MORPHOLOGY AND STRUCTURE

The Enterobacteriaceae are among the largest bacteria, measuring 2 to 4 $\mu$m in length and 0.4 to 0.6 $\mu$m in width, with parallel sides and rounded ends. Forms range from large coccobacilli to elongated, filamentous rods. The organisms do not form spores or demonstrate acid fastness.

Rods are large

The cell wall, cell membrane, and internal structures are morphologically similar for all Enterobacteriaceae, and follow the cell plan described in Chapter 2 for Gram-negative bacteria. Components of the cell wall and surface, which are antigenic, have been extensively studied in some genera and form the basis of systems dividing species into serotypes (Fig 21–1). The outer membrane lipopolysaccharide (LPS) is called the **O antigen.** Its antigenic specificity is determined by the composition of the sugars that form the long terminal polysaccharide side chains linked to the core polysaccharide and lipid A. Cell surface polysaccharides may form a well-defined capsule or an amorphous slime layer and are termed the **K antigen** (from the Danish Kapsel, capsule). Motile strains have protein peritrichous flagella, which extend well beyond the cell wall and are called the **H antigen.** Many of the Enterobacteriaceae have surface pili, which are antigenic proteins but not yet part of any formal typing scheme.

O = LPS

K = polysaccharide capsule

H = flagellar protein

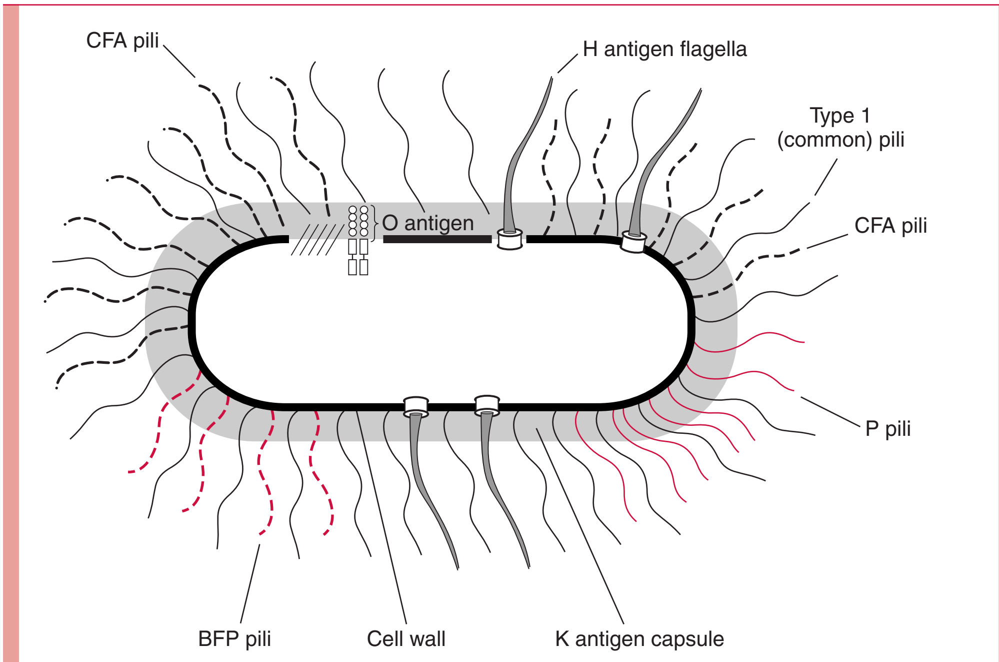


FIGURE 21–1
Antigenic structure of *Escherichia coli*. The O antigen is contained in the repeating polysaccharide units of the lipopolysaccharide (LPS) in the outer membrane of the cell wall. The H antigen is flagellar protein. The K antigen is the polysaccharide capsule present in some strains. Most *E. coli* have type 1 (common) hair-like pili extending from the surface. Some *E. coli* have specialized P, colonization factor antigens (CFAs), or bundle-forming pili (BFP), as well as type 1 pili.

## GROWTH AND METABOLISM

Facultative growth is rapid

Enterobacteriaceae grow readily on simple media, often with only a single carbon energy source. Growth is rapid under both aerobic and anaerobic conditions, producing 2- to 5-mm colonies on agar media and diffuse turbidity in broth after 12 to 18 hours of incubation. All Enterobacteriaceae ferment glucose, reduce nitrates to nitrites, and are oxidase negative.

## CLASSIFICATION

Biochemical characteristics establish species

Antigenic characters define serotypes within species

Genus and species designations are based on phenotypic characteristics, such as patterns of carbohydrate fermentation, and amino acid breakdown. The O, K, and H antigens are used to further divide some species into multiple **serotypes.** These types are expressed with letter and number of the specific antigen, such as *Escherichia coli* **O**157:**H**7, the cause of numerous food-borne outbreaks. These antigenic designations have been established only for the most important species and are limited to the structures at hand. For example, many species lack capsules and/or flagella. In recent years, DNA and RNA homology data have been used to validate these relationships and establish new ones. The genera containing the species most virulent for humans are *Escherichia, Shigella, Salmonella, Klebsiella,* and *Yersinia.* Other less common medically important genera are *Enterobacter, Serratia, Proteus, Morganella,* and *Providencia.*

## TOXINS

All have LPS

In addition to the **LPS endotoxin** common to all Gram-negative bacteria, some Enterobacteriaceae also produce **protein exotoxins,** which act on host cells by damaging membranes, inhibiting protein synthesis, or altering metabolic pathways. The end result of these actions may be cell death (cytotoxin) or a physiologic alteration, the net effect of which depends on the function of the affected cell. For example, enterotoxins act on intestinal enterocytes, causing the net secretion of water and electrolytes into the gut to produce diarrhea. Although these toxins are most strongly associated with *E. coli, Shigella,* and *Yersinia,* others with the same or very similar actions have now been discovered in other species. When found in another species, the toxin may differ by a few amino acids in structure and in genetic regulation but has the same basic action on host cells. Details of these toxins are discussed below in relation to their prototype species.

Cytotoxins kill cells

Enterotoxins cause diarrhea

# DISEASES CAUSED BY ENTEROBACTERIACEAE

## EPIDEMIOLOGY

Most Enterobacteriaceae are primarily colonizers of the lower gastrointestinal tract of humans and animals. Many species survive readily in nature and live freely anywhere water and minimal energy sources are available. In humans, they are the major facultative components of the colonic bacterial flora and are also found in the female genital tract and as transient colonizers of the skin. Enterobacteriaceae are scant in the respiratory tract of healthy individuals; however, their numbers may increase in hospitalized patients with chronic debilitating diseases. *E. coli* is the most common species of Enterobacteriaceae found among the indigenous flora, followed by *Klebsiella, Proteus,* and *Enterobacter* species. *Salmonella* and *Shigella* species are not considered members of the normal flora, although carrier states can exist. *Shigella* and *Salmonella* serotype Typhi are strict human pathogens.

Present in nature and the intestinal tract

*Shigella* and *S. typhi* are found only in humans

## PATHOGENESIS

### Opportunistic Infections

Enterobacteriaceae are often poised to take advantage of their common presence in the environment and normal flora to produce disease when they gain access to normally sterile body sites. Surface structures such as pili are known to aid this process for some species and surely do for many others. Once in deeper tissues, their ability to persist and cause injury is little understood except for the action of LPS endotoxin and the species known to produce exotoxins or capsules. The prototype opportunistic infection is the UTI, in which Enterobacteriaceae gain access to the urinary bladder due to minor trauma or instrumentation. Strains able to adhere to uroepithelial cell can persist and multiply in the nutrient-rich urine, sometimes spreading through the ureters to the renal pelvis and kidney (pyelonephritis). Likewise, mucosal or skin trauma can allow access to soft tissues and aspiration to the lung when the relevant site is colonized with Enterobacteriaceae.

Colonization presents opportunity when defense barriers open

UTI follows access and adherence to bladder mucosa

### Intestinal Infections

*Salmonella, Shigella, Yersinia enterocolitica,* and certain strains of *E. coli* are able to produce disease in the intestinal tract. These intestinal pathogens have invasive properties or virulence factors such as cytotoxins and enterotoxins, which correlate with the type of diarrhea they produce. In general, the invasive and cytotoxic strains produce an inflammatory diarrhea called **dysentery** with white blood cells (WBCs) and/or blood in the stool. The enterotoxin-producing strains cause a **watery diarrhea** in which fluid loss is the primary pathophysiologic feature. For a few species, the intestinal tract is the portal of entry, but the disease is systemic due to spread of bacteria to multiple organs. **Enteric (typhoid) fever** caused by *Salmonella* serotype Typhi is the prototype of this form of infection.

Cell destruction causes dysentery

Enterotoxins cause watery diarrhea

Enteric fever is a systemic illness

### Regulation of Virulence

In addition to adherence pili, LPS, and exotoxins, the Enterobacteriaceae produce a myriad of other virulence factors in order to cause disease. Many of them are deployed in a

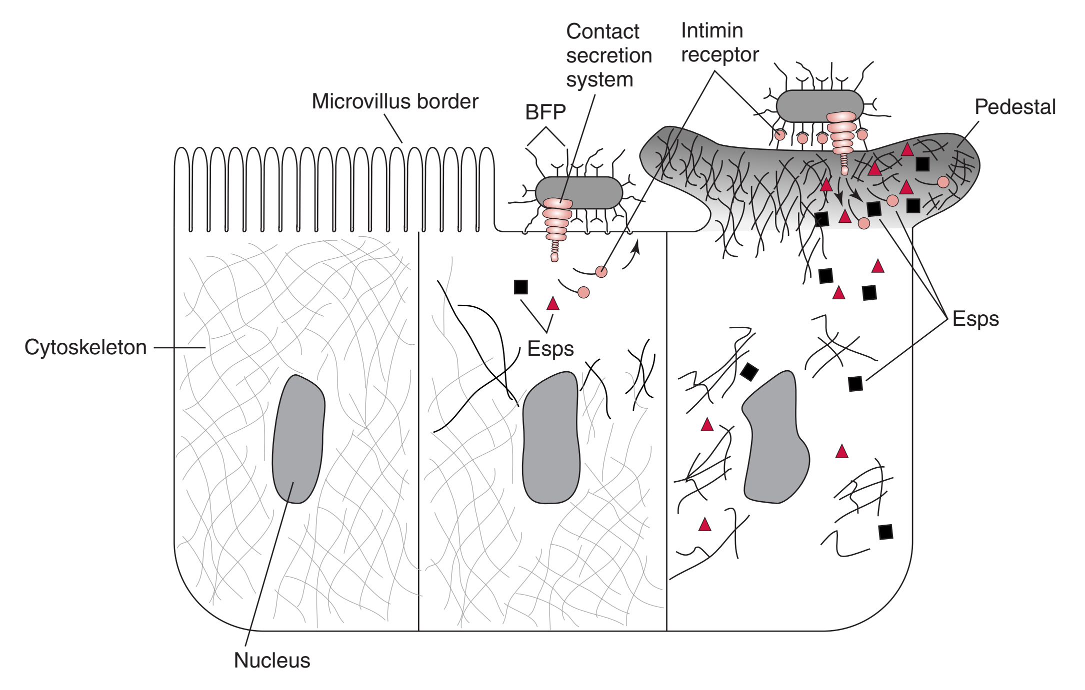


FIGURE 21–2
Enteropathic *Escherichia coli* (EPEC) contact secretion system. **Left.** An enterocyte is shown with a microvillus border and a delicate supporting cytoskeleton. **Middle.** An EPEC has attached to the cell surface by binding of the bundle-forming pili to receptors on the host cell surface. A contact secretion apparatus (see Figure 3–8) has been inserted into the cell and is exporting secretion proteins (Esps) into the cytoplasm. One of these is the receptor for intimin. **Right.** The intimin receptor has been inserted below the host cell membrane and is now mediating tight binding to the surface. The other Esps have disrupted multiple cellular functions, including the structure of the cytoskeleton. Cytoskeleton elements have been concentrated to form a pedestal cradling the EPEC.

complex and sequential fashion in response to environmental cues (temperature, iron, calcium) or as yet unknown factors.

Some bacteria have **contact secretion systems** (Fig 21–2) that target human cells by literally delivering a syringe-like injection of virulence factors into the cytoplasm of host cells.

Virulence genes are organized into gene clusters

Expression may be stimulated by environmental cues

PAIs contain DNA from another bacterium

The genes for these factors, located in the chromosome, plasmids, or both, are controlled by interactive regulators that seem to produce each virulence factor exactly when it is needed. The genes themselves are often organized into clusters that include the genes for the effector molecules as well as their regulatory proteins. This is particularly true for complex characteristics like invasiveness which involve multiple sequential steps. Some of these gene clusters are called **pathogenicity islands** (PAIs) because their overall genetic makeup is foreign enough to the rest of the genome of the organism that they appear to have been acquired from another bacterium in the genetically distant past. In particular, PAIs are associated with contact secretion systems where they contain the structural genes for the injection apparatus as well as the virulence factors injected.

## IMMUNITY

Immunity is short-lived

Little is understood about immunity to the broad range of opportunistic infections caused by Enterobacteriaceae. Antibody directed against an LPS core antigen has been shown to provide a degree of protection against Gram-negative endotoxemia, but the diversity of antigens and virulence factors among the Enterobacteriaceae is too great to expect broad immunity. Immunity to intestinal infection is generally short-lived and will be discussed where it is relevant to specific intestinal pathogens.

# ENTEROBACTERIACEAE: CLINICAL ASPECTS

## MANIFESTATIONS

The Enterobacteriaceae produce the widest variety of infections of any group of microbial agents, including two of the most common infectious states, UTI and acute diarrhea. UTIs are manifested by dysuria and urinary frequency when infection is limited to the bladder, with the addition of fever and flank pain when the infection spreads to the kidney. Enterobacteriaceae are by far the most common cause of UTIs, and the most common species involved is *E. coli.* The features of UTIs are discussed in more detail in Chapter 66.

UTI and acute diarrhea are most common

## DIAGNOSIS

Culture is the primary method of diagnosis; all Enterobacteriaceae are readily isolated on routine media under almost any incubation conditions. Special indicator media such as MacConkey agar are commonly used in primary isolation to speed separation of the many species. For example, the common pathogens *E. coli* and *Klebsiella* typically ferment lactose rapidly, producing acid (pink) colonies on MacConkey agar, whereas the intestinal pathogens *Salmonella* and *Shigella* do not. Separation of the intestinal pathogens from all the other Enterobacteriaceae present in stool requires the use of highly selective media designed solely for this purpose. They will be discussed as they relate to individual pathogens.

Improved understanding of the genetic and molecular basis for virulence has led to the development of direct nucleic acid and immunodiagnostic techniques for direct detection of toxin, adhesin, or invasin genes in clinical material (eg, stool). These methods are still too expensive for use in clinical laboratories but are of extraordinary value in epidemiologic work and clinical research.

Culture on MacConkey agar demonstrates lactose fermentation

Selective media required for *Salmonella* and *Shigella* in stools

## TREATMENT

Antimicrobial therapy is crucial to the outcome of infections with members of the Enterobacteriaceae. Unfortunately, combinations of chromosomal and plasmid-determined resistance (see Chapter 14) render them the most variable of all bacteria in susceptibility to antimicrobial agents. They are usually resistant to high concentrations of penicillin G, erythromycin, and clindamycin, but may be susceptible to the broader-spectrum $\beta$-lactams, aminoglycosides, tetracycline, chloramphenicol, sulfonamides, quinolones, nitrofurantoin, and the polypeptide antibiotics. Because the probability of resistance varies among genera and in different epidemiologic settings, the susceptibility of any individual strain must be determined by in vitro tests. Typical frequencies of resistance for some of the more common Enterobacteriaceae appear in Table 13–1.

Susceptibility to antimicrobials is highly variable

# *ESCHERICHIA COLI*

# BACTERIOLOGY

## CLASSIFICATION

Most strains of *E. coli* ferment lactose rapidly and produce indole. These and other biochemical reactions are sufficient to separate it from the other species. There are over 150 distinct O antigens and a large number of K and H antigens, all of which are designated

Hundreds of serotypes are possible

by number. The antigenic formula for serotypes is described by linking the letter (O, K, or H) and number of the antigens present (eg, **O**111:**K**76:**H**7).

## PILI

Type 1 pili bind mannose

Pili (also called fimbriae) are frequently present on the surface of *E. coli* strains. Research has shown that some of these structures play a role in virulence as mediators of attachment to human epithelial surfaces. Pili show marked tropism for different epithelial cell types, which is determined by the availability of their specific receptor on the host cell surface. Most *E. coli* express **type 1** (common) pili. Type 1 pili bind to the D-mannose residues commonly present on epithelial cell surfaces and thus mediate binding to a wide variety of cell types.

P pili bind uroepithelial cells

CFA and BFP pili bind enterocytes

More specialized pili are found in subpopulations of *E. coli*. **P pili** (also called Pap or Gal–Gal) bind to digalactoside (Gal–Gal) moieties present on certain mammalian cells, including uroepithelial cells and erythrocytes of the P blood group. Other pili bind to intestinal cells and have their own set of specificities. Those binding to human enterocytes are called **colonization factor antigens** (CFAs) or **bundle-forming pili** (BFP), depending on the pathogenic type of *E. coli* involved and possibly the cell type in the gastrointestinal tract. The specific binding receptors for the enterocyte binding pili are not known.

Type 1 has on–off switch

The genetics of pilin expression is complex. The genes are organized into multicistronic clusters that encode structural pilin subunits and regulatory functions. Pili of different types may coexist on the same bacterium, and their expression may vary under different environmental conditions. Type 1 pilin expression can be turned "on" or "off" by inversion of a chromosomal DNA sequence containing the promoter responsible for initiating transcription of the pilin gene. Other genes control the orientation of this switch.

## TOXINS

*E. coli* can produce every kind of toxin found among the Enterobacteriaceae. These include a pore-forming cytotoxin, inhibitors of protein synthesis, and a number of toxins that alter messenger pathways in host cells.

$\alpha$-hemolysin is pore-forming cytotoxin

The **$\alpha$-hemolysin** is a pore-forming cytotoxin that inserts into the plasma membrane of a wide range of host cells in a manner similar to streptolysin O (see Chapter 17) and *Staphylococcus aureus* $\alpha$-toxin (see Chapter 16). The toxin causes leakage of cytoplasmic contents and eventually cell death.

Shiga toxin is produced by *Shigella* and *E. coli*

Inhibits protein synthesis by ribosomal binding

**Shiga toxin** is named for the microbiologist who discovered *Shigella dysenteriae,* and this toxin was once believed to be limited to that species. It is now recognized to exist in at least two molecular forms released by multiple *E. coli* and *Shigella* strains on lysis of the bacteria. In the years following the discovery of this toxin, the term Shiga toxin was reserved for the original toxin, and others were called Shiga-like. In this discussion, the term Shiga toxin will be used for all the molecular variants that have the same mode of action. Shiga toxins are of the AB type. The B unit directs binding to a specific glycolipid receptor ($Gb_3$) present on eukaryotic cells and is internalized in an endocytotic vacuole. Inside the cell, the A subunit crosses the vacuolar membrane in the trans-Golgi network, exits to the cytoplasm, and enzymatically modifies 28S-ribosomal RNA of the 60S-ribosomal subunit by removing an adenine base. This prevents the elongation-factor-1–dependent binding of amino acyl tRNA to the ribosome blocking protein synthesis, leading to cell death.

LT ADP-ribosylates G protein

Adenylate cyclase stimulation is similar to cholera toxin

**Labile toxin (LT)** is also an AB toxin. Its name relates to the physical property of heat lability, which was important in its discovery, and contrasts with the heat-stable toxin described below. The B subunit binds to the cell membrane, and the A subunit catalyzes the ADP-ribosylation of a regulatory G protein located in the membrane of the intestinal epithelial cell. This inactivation of part of the G protein causes permanent activation of the membrane-associated adenylate cyclase system and a cascade of events, the net effect of which depends on the biological function of the stimulated cell. If the cell is an enterocyte, the result is the stimulation of chloride secretion out of the cell and the blockage of NaCl absorption. The net effect is the accumulation of water and electrolytes into the bowel lumen. The structure and biological effect of LT is very similar to cholera toxin, which is described in Chapter 22.

**Stable toxin (ST)** toxin is a small (17- to 18-amino acid) peptide that binds to a glycoprotein receptor, resulting in the activation of a membrane-bound guanylate cyclase. The subsequent increase in cyclic GMP concentration causes an LT-like net secretion of fluid and electrolytes into the bowel lumen.

ST stimulates guanylate cyclase

# *E. coli* OPPORTUNISTIC INFECTIONS

## URINARY TRACT INFECTION (UTI)

CLINICAL CAPSULE

The term UTI encompasses a range of infections from simple cystitis involving the bladder to full-blown infection of the entire urinary tract, including the renal pelvis and kidney (pyelonephritis). The primary feature of cystitis is frequent urination, which has a painful burning quality. In pyelonephritis, symptoms include fever, general malaise, and flank pain in addition to the frequent urination. Cystitis is usually self-limiting, but infection of the upper urinary tract carries a risk of spread to the bloodstream. It is the leading cause of Gram-negative sepsis and septic shock.

### Epidemiology

*E. coli* accounts for more than 90% of the more than 7 million cases of cystitis and 250,000 of pyelonephritis estimated to occur in otherwise healthy individuals every year in the United States. UTIs are more common in women, 40% of whom have an episode in their lifetime, usually when they are sexually active. The reservoir for these infections is the patient's own intestinal *E. coli* flora, which contaminate the perineal and urethral area. In individuals with urinary tract obstruction or instrumentation, environment sources assume some importance.

Perineal flora is reservoir of common cystitis

### Pathogenesis

Relatively minor trauma or the mechanical effect of sexual intercourse have been shown to allow bacteria access to the bladder. In most instances, these bacteria are purged by the flushing action of voiding. Factors that violate bladder integrity (urinary catheters) or that obstruct urine outflow (enlarged prostate) are also associated with infection. However, this cannot be the whole story; fewer than 10 *E. coli* serotypes account for the majority of UTI cases, and these UTI serotypes are not the dominant ones in the fecal flora.

Minor trauma admits *E. coli* to the bladder

The ability of uropathic *E. coli* (UPEC) to produce UTI is related to general virulence factors such as $\alpha$-hemolysin, together with pili-mediated adherence to uroepithelial cells. The percentage of *E. coli* with P pili increases from 20% in the fecal flora to 70% in pyelonephritis isolates. Asymptomatic bacteriuria and cystitis isolates fall in between. The digalactoside receptor for P is present on uroepithelial cells, to which the bacteria bind avidly, particularly in the upper urinary tract. By aiding in periurethral colonization as the prelude to bladder access, type 1 pili are important as well. In addition, type 1 pili are essential for attachment to urinary epithelium in the urinary bladder where they appear to keep their invertible segment in the "on" position. They are not involved in pyelonephritis where P pili are more important. Antipilin antibody blocks adherence in experimental systems, suggesting that immunization could be an approach to UTI prevention. The pathogenic features that allow *E. coli* to play such a prominent role in this disease are illustrated in Figure 21–3.

P pili adhere to digalactoside receptor

Upper tract is favored by P pili

Type 1 pili are important in the bladder

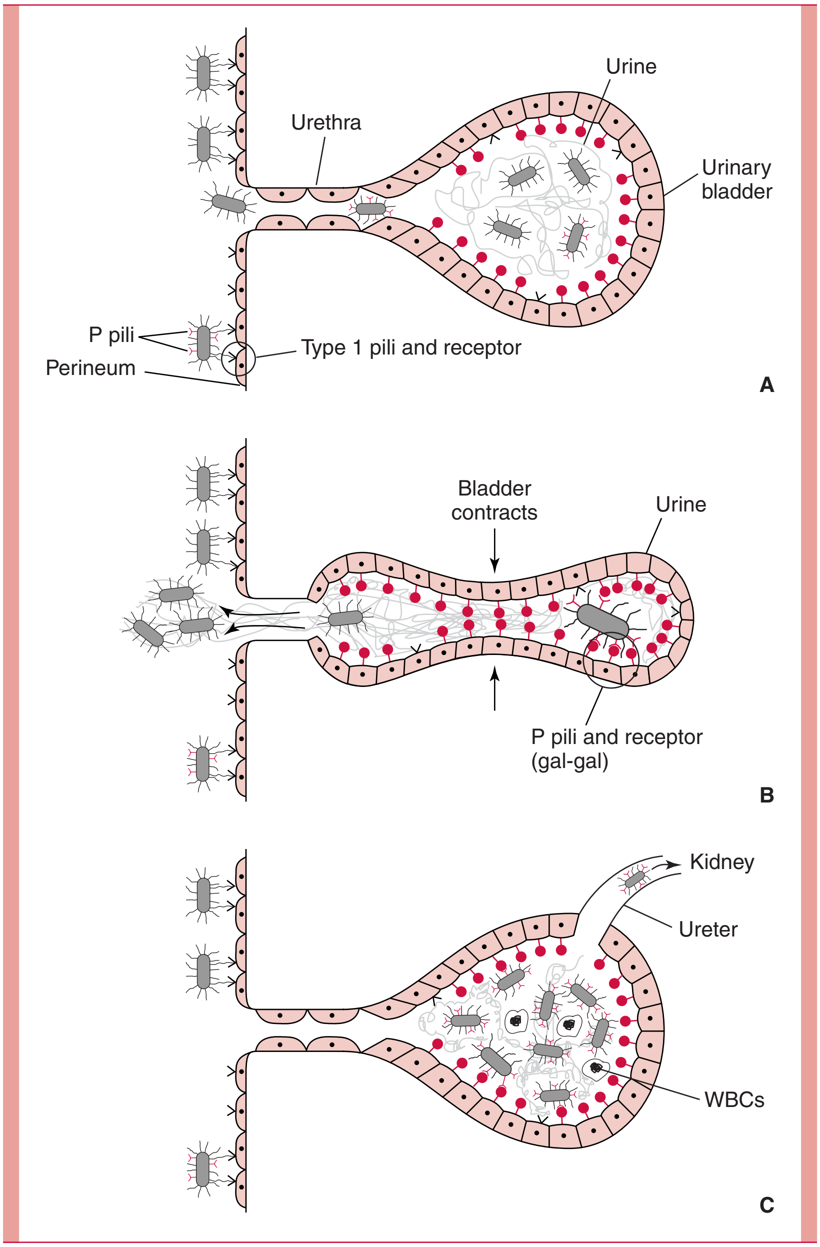


FIGURE 21–3
Urinary tract infection due to *Escherichia coli.* The urinary bladder, perineal mucosa, and short female urethra are shown. *E. coli* from the nearby rectal flora have colonized the perineum, utilizing binding by type 1 (common) pili. *E. coli* with P pili are also present but are of no use at this site. **A.** A few *E. coli* have gained access to the bladder due to mechanical disruptions, such as sexual intercourse or instrumentation (catheters). Note that receptors for the P pili not present on the perineal mucosa are found on the surface of bladder mucosal cells. **B.** During voiding, the bladder has expelled the *E. coli,* which only have type 1 pili. The P pili–containing bacteria remain behind due to the strong binding to the P (gal-gal) receptor. **C.** The remaining *E. coli* have multiplied and are causing a urinary tract infection (cystitis) with inflammation and hemorrhage. In some cases, the bacteria ascend the ureter to cause pyelonephritis.

## OTHER OPPORTUNISTIC INFECTIONS

### Meningitis

Infection from vaginal flora such as group B streptococci

K1 capsular polysaccharide is identical to meningococcus

*E. coli* is one of the most common causes of neonatal meningitis, many features of which are similar to group B streptococcal disease (see Chapter 17). The pathogenesis involves vaginal *E. coli* colonization of the infant via ruptured amniotic membranes or during childbirth. Failure of protective maternal immunoglobulin M (IgM) antibodies to cross the placenta and the special susceptibility of newborns surely plays a role. Fully 75% of cases are caused by strains possessing the K1 capsular polysaccharide that contains sialic acid and is structurally identical to the group B polysaccharide of *Neisseria meningitidis,* another cause of meningitis. There is some evidence that these strains have a type of pili with the property of adherence to brain microvascular endothelial cells.

Non-UTI infections require some breach of defenses

With the exception of UTIs, extraintestinal *E. coli* infections are uncommon unless there is a significant breach in host defenses. Opportunistic infection may follow mechanical damage such as a ruptured intestinal diverticulum, trauma, or involve a generalized impairment of immune function. The virulence factors involved are likely the same as with UTI (eg, pili, α-hemolysin) but have been less specifically studied. Failure of local control of infection can lead to spread and eventually Gram-negative septic shock. A significant proportion of blood isolates have the K1 surface polysaccharide. The particular diseases that result depend on the sites involved and include many of the syndromes covered in Chapters 59 to 72.

## *E. coli* INTESTINAL INFECTIONS

CLINICAL CAPSULE

Diarrhea is the universal finding with *E. coli* strains that are able to cause intestinal disease. The nature of the diarrhea varies depending on the pathogenic mechanism. Enterotoxigenic and enteropathogenic strains produce a watery diarrhea, the enterohemorrhagic strains produce a bloody diarrhea, and the enteroinvasive strains may cause dysentery with blood and pus in the stool. The diarrhea is usually self-limiting after only 1 to 3 days. The enterohemorrhagic *E. coli* are an exception, with life-threatening manifestations outside the gastrointestinal tract due to Shiga toxin production.

Diarrhea-causing *E. coli* are conveniently classified according to their virulence properties as **enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC),** or **enteroaggregative (EAEC).** Each group causes disease by a different mechanism, and the resulting syndromes usually differ clinically and epidemiologically. For example, human ETEC and EIEC strains infect only humans. Food and water contaminated with human waste and person-to-person contact are the principal means of infection. A summary of the pathogenesis of infection, clinical syndromes, and epidemiology of infection for each enteropathogen is shown in Table 21–1.

Multiple pathogenic mechanisms have their own epidemiologic and clinical features

### ENTEROTOXIGENIC *E. coli* (ETEC)

### Epidemiology

ETEC are the most important cause of traveler's diarrhea in visitors to developing countries. ETEC also produce diarrhea in infants native to these countries, where they are a leading cause of morbidity and mortality during the first 2 years of life. Repeated bouts of diarrhea caused by ETEC and other infectious agents are an important cause of growth retardation, malnutrition, and developmental delay in the third world countries where ETEC are endemic. ETEC disease is rare in industrialized nations.

Traveler's diarrhea affects children of developing countries

Transmission is by consumption of food and water contaminated by human cases or convalescent carriers. Uncooked foods such as salads or marinated meats and vegetables are associated with the greatest risk. Direct person-to-person transmission is unusual, because the infecting dose is high. Animals are not involved in ETEC disease.

Oral ingestion of uncooked foods requires high dose for disease

### Pathogenesis

ETEC diarrhea is caused by strains of *E. coli* that produce LT and/or ST enterotoxins in the small intestine. Strains that elaborate both LT and ST cause more severe illness. Adherence to surface microvilli mediated by the CFA class of pili is essential for the efficient delivery of toxin to the target enterocytes. The genes encoding the ST, LT, and the CFA pili are borne in plasmids. A single plasmid can carry all three sets of genes. The bacteria remain on the surface, where the adenylate cyclase–stimulating action of the toxin(s) creates the flow of water and electrolytes from the enterocyte into the intestinal lumen. The mucosa becomes hyperemic but is not injured in the process. There is no invasion or inflammation.

LT and/or ST cause fluid outpouring in small intestine

CFA pili are required

TABLE 21–1

## Characteristics of Pathogenic Enterobacteriaceae

| | | Surface | | Virulence Factors | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Diagnostic Antigens | Adherence | Capsule | Exotoxin(s) | Pathogenic Lesions | Secreted Proteins[a] | Genetics | Transmission | Disease |
| ***Escherichia coli*** | O, H, K | | | | | | | | |
| Common | More than 150 types | Type 1[b] pili | K1 | α-Hemolysin | Inflammation | | | Adjacent flora | Opportunistic |
| Uropathic UPEC | | Type 1[b] P pili | | α-Hemolysin | Inflammation | | | Fecal flora, ascending | Urinary tract |
| Enterotoxigenic (ETEC) | | CFA pili | | LT, ST | Hypersecretion | | Plasmid (CFA, LT, ST) | Fecal–oral | Watery diarrhea (travelers) |
| Enteropathogenic (EPEC) | | Bundle-forming pili, Intimin | | | A/E, small intestine | Esp | PAI | Fecal–oral | Watery diarrhea |
| Enteroinvasive (EIEC) | | | | | Invasion, inflammation, ulcers | | Large plasmid, PAI | Fecal–oral | Dysentery |
| Enterohemorrhagic (EHEC) | 0157;H7 | Intimin | | Shiga toxin | A/E, colon, hemorrhage | Esp | PAI | Fecal–oral direct, low dose, cattle | Bloody diarrhea, HUS |
| Enteraggregative (EAEC) | | | | | Adherent biofilm | | | | Mucoid, watery diarrhea |
| ***Shigella*** | O serogroups | | | | | | | | |
| *S. dysenteriae* | A(10 types) | | | Shiga toxin (A1 potent) | Invasion, inflammation, colonic ulcers | Ipa | Large plasmid, PAI | Fecal–oral, direct, low dose | Dysentery (severe), HUS |
| *S. flexneri* | B (6 types) | | | Shiga toxin (variable) | Invasion, inflammation, colonic ulcers | Ipa | Large plasmid, PAI | Fecal–oral, direct, low dose | Dysentery, HUS |
| *S. boydii* | C (15 types) | | | Shiga toxin (variable) | Invasion, inflammation, colonic ulcers | Ipa | Large plasmid, PAI | Fecal–oral, direct, low dose | Dysentery, HUS |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *S. sonnei* | D | | | Shiga toxin (variable) | Invasion, inflammation, colonic ulcers | Ipa | Large plasmid, PAI | Fecal–oral, direct, low dose | Dysentery, HUS |
| ***Salmonella enterica*** | O, $H_1$, $H_2$, K | | | | | | | | |
| Serotypes | More than 2000 | Pili | | | Ruffles, invasion, inflammation | Inv, Spa, others | PAI | Fecal–oral, animals and humans | Gastroenteritis, sepsis |
| Typhi | O group D | Pili | Vi | | Macrophage survival, RES growth | As in serotypes[c] | PAI | Fecal–oral, moderate dose, humans only | Enteric (typhoid) fever |
| ***Yersinia*** | O, H | | | | | | | | |
| *Y. pestis* | | Invasin | Protein | Protease, fibrinolysin | RES growth, bacteremia, pneumonia | Yop | PAI | Rats, flea bite, aerosol (human) | Plague |
| *Y. pseudotuberculosis* | 10 types | Invasin | | | RES growth, microabscesses | Yop | PAI | Fecal–oral, animal | Mesenteric adenitis |
| *Y. enterocolitica* | More than 50 types | Invasin | | | RES growth, microabscesses | Yop | PAI | Fecal–oral, animals | Mesenteric adenitis, enteric fever |
| ***Klebsiella*** | 70 capsular types | Pili | Polysaccharide | | | | | Adjacent flora | Opportunistic, pneumonia |
| ***Enterobacter*** | | | | | | | | Adjacent flora | Opportunistic |
| ***Serratia*** | | | | | | | | Adjacent flora | Opportunistic |
| ***Citrobacter*** | | | | | | | | Adjacent flora | Opportunistic |
| ***Proteus*** | | | | | | | | Adjacent flora | Opportunistic |

*Abbreviations:* A/E, attaching and effacing lesion; CFA, colonizing factor antigen; Esp, *E. coli*–secreted protein; HUS, hemolytic uremic syndrome; lpa, invasion protein antigen; LT, labile toxin; PAI, pathogenicity island; RES, reticuloendothelial system; ST, stable toxin; Yop, *Yersinia* outer membrane protein.

[a] Delivered by type III secretion system.

[b] Bind to mannose.

[c] No animal model, presumed to be similar to *S. enterica* serotypes.

## Immunity

sIgA to LT and CFAs may provide some protection

Although there can be more than one episode of diarrhea, infections with ETEC can stimulate immunity. Travelers from industrialized nations have a much higher attack rate than adults living in the endemic area. This natural immunity is presumably mediated by sIgA specific for LT and CFAs. The small ST peptides are nonimmunogenic. The disease is of very low incidence in breast-fed infants, underscoring the protective effect of maternal antibody and the importance of transmission by contaminated food and water.

# ENTEROPATHOGENIC *E. coli* (EPEC)

## Epidemiology

Nursery outbreaks and endemic diarrheas occur in developing world

EPEC strains were first identified as the cause of explosive outbreaks of diarrhea in hospital nurseries in the United States and Great Britain during the 1950s. The disease seems to have disappeared in industrialized nations, although it may be underestimated due to the difficulty of diagnosis. In developing countries throughout the world, EPEC account for up to 20% of diarrhea in bottle-fed infants younger than 1 year of age. The reservoir is infant cases and adult carriers with transmission by the fecal–oral route. Nursery outbreaks demonstrate the importance of spread by fomites, which suggests that the infecting dose for infants is low. Documented adult cases have usually been in circumstances where the number of organisms ingested was very large.

## Pathogenesis

A/E lesions involve modification of cytoskeleton

Secretion system injects receptor for intimin of EPEC

EPEC initially attach to enterocytes using pili of the BFP type to form clustered microcolonies on the enterocyte cell surface. The lesion then progresses with effacement of the microvilli and changes in the cell morphology including the production of dramatic "pedestals" with the EPEC bacterium at their apex. The combination of these actions is called the **attachment and effacing (A/E)** lesion (Fig 21–4). The many steps involved in the formation of the A/E lesion are genetically controlled in a PAI, which includes the genes for the major attachment protein, **intimin,** and a contact secretion system. The secretion system injects at least five ***E. coli* secretion proteins (Esps)** into the host cell cytoplasm including, remarkably, the receptor for intimin. The other *E. coli* secretion proteins perturb intracellular signal transduction pathways, one effect of which is the induction of modifications in enterocyte cytoskeleton proteins (actin, talin). The cytoskeleton accumulates beneath the attached bacteria to form the pedestals and complete the A/E lesion. Exactly how this leads to diarrhea is not known, but the change from the normal microvillus border to the A/E must disrupt intestinal absorptive functions.

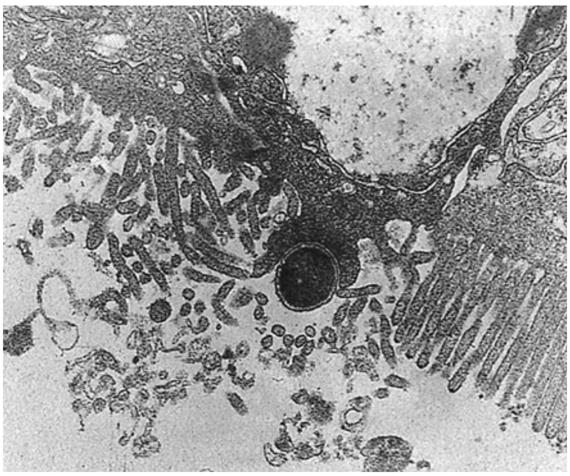

FIGURE 21–4
Enteropathogenic *Escherichia coli* (EPEC) attachment to epithelial cells. The EPEC are attaching to and effacing the microvilli on the epithelial cell surface. The cell's filamentous actin is rearranged at the attachment point

## Immunity

In endemic areas, EPEC can be isolated often from the stool of asymptomatic adults, but unlike ETEC, these strains do not seem to cause traveler's diarrhea in individuals new to the area. This casts doubt on whether adults have acquired immunity or resistance based on physiologic factors.

Little evidence for immunity

# ENTEROHEMORRHAGIC *E. coli* (EHEC)

## Epidemiology

EHEC disease and the accompanying **hemolytic uremic syndrome (HUS)** are the result of consumption of products from animals colonized with EHEC strains. It is also clear from secondary cases in families during outbreaks that person-to-person transmission also occurs. This disease occurs more in developed rather than in developing countries.

Consumption of contaminated animal products is the main source

EHEC was first recognized in the early 1980s when outbreaks of HUS (hemolytic anemia, renal failure, and thrombocytopenia) were linked to a single *E. coli* serotype, O157:H7. Since then EHEC disease has emerged as an important cause of **bloody diarrhea** in industrialized nations and retained a remarkable but not exclusive relationship with the O157:H7 serotype, particularly in North America. Regional and national outbreaks associated with unpasteurized juices and hamburger have caught the attention of the public, the press, and the government.

Bloody diarrhea and HUS are linked to O157:H7

The emergence of EHEC is related to its virulence (see below), low infecting dose, common reservoir (cattle), and changes in the modern food processing industry that provide us with fresher meat (and bacteria). The low infecting dose, estimated at 100 to 200 organisms, is particularly important. This is a level where food need not come directly from the infected animal, only be contaminated by it. For example, large modern meat processing plants can mix EHEC from colonized cattle at one ranch into beef from hundreds of other farms and quickly ship it all over the country. Therefore, the worst outbreaks have been seen in countries with the most advanced food production systems. If the organisms are ground into hamburger, an infecting dose of EHEC may remain even after cooking if the meat is left rare in the middle. Unpasteurized milk carries an obvious risk but fruits and vegetables have also been the source for EHEC infection. In these instances the EHEC from the manure of cattle grazing nearby has contaminated these products in the field. The bacterial dose from a few "drop" apples (those picked up from the ground) included in a batch of cider has been enough to cause disease.

Low infecting dose facilitates transmission

Modern meat processing facilitates widespread outbreaks

Unpasteurized beverages are another risk

## Pathogenesis

The distinguishing feature of the EHEC is the production of both Shiga toxins and the A/E lesions described above for EPEC. Another difference between EHEC and EPEC is that EHEC primarily attacks the colon while EPEC infects the small intestine. The multiple extraintestinal features such as HUS appear to be the result of circulating Shiga toxin. The interaction of EHEC with enterocytes is much the same as EPEC, except the EHEC strains do not form localized microcolonies on the mucosa. The outer membrane protein intimin mediates adherence and the contact secretion system injects the *E. coli* secretion proteins, which cause alterations in the host cytoskeleton. The genes for these properties are also found in a PAI.

Produce both Shiga toxin and A/E lesions

Circulating Shiga toxin leads to HUS

The A/E features alone are sufficient to cause nonbloody diarrhea. Shiga toxin production causes capillary thrombosis and inflammation of the colonic mucosa, leading to a hemorrhagic colitis. Although it has not been detected in the blood of human cases, Shiga toxin is presumed to be absorbed across the denuded intestinal mucosa. Circulating Shiga toxin binds to renal tissue where its glycoprotein receptor is particularly abundant, causing glomerular swelling and the deposition of fibrin and platelets in the microvasculature. How Shiga toxin causes hemolysis is less clear; perhaps the erythrocytes are simply damaged as they attempt to traverse the occluded capillaries. The strong association between EHEC disease and the O157:H7 serotype suggests that EHEC are more than just Shiga toxin–producing EPEC. The O157:H7 strains invariably have a large plasmid which may

Shiga toxin capillary thrombosis and inflammation have a hemorrhagic component

O157:H7 strains differ from EPEC in more than Shiga toxin contain other virulence genes. Cases and outbreaks caused by Shiga toxin–producing *E. coli* of other serotypes may be on the rise and are common in some countries. How they differ from O157:H7 EPEC remains to be seen.

## ENTEROINVASIVE *E. coli* (EIEC)

EIEC closely resemble *Shigella*

Virtually all aspects of EIEC disease are identical to *Shigella* (see below), which underscores the close relationship of the *Shigella* and *Escherichia* genera. Epidemiologically, EIEC infections are essentially restricted to children under 5 years of age living in developing nations. The occasional documented outbreaks in industrialized nations are usually linked to contaminated food or water. This lower incidence of person-to-person transmission correlates with the observation that the infecting dose for EIEC is higher than it is for *Shigella.* Humans are the only known reservoir.

## ENTEROAGGREGATIVE *E. coli* (EAEC)

Adherence alone may create a biofilm

EAEC is associated with a protracted (>14 days) mucoid, watery diarrhea in infants and children in developing countries. The EAEC strains are defined on the basis of the pattern the bacteria make (eg, localized, diffuse, stacked) when adhering to cultured mammalian cells. Even though EAEC adheres tightly to the intestinal mucosa, the A/E lesions of the EPEC and EHEC are not present. The pathogenesis of diarrhea is not clearly understood but may involve the ability to form a mucus–bacteria biofilm on the intestinal surface. Inflammatory cells are not seen.

# *E. coli* INFECTIONS: CLINICAL ASPECTS

## MANIFESTATIONS

### Opportunistic Infections

Dysuria and frequency are features of UTIs

The most common symptoms of *E. coli* UTI are dysuria and urinary frequency and do not differ significantly in character from those produced by the other less common Gram-negative urinary pathogens discussed in Chapter 66. If the infection ascends the ureters to produce pyelonephritis, fever and flank pain are common and bacteremia may develop. Although *E. coli* may have enhanced virulence in the production of pneumonia as well as soft tissue and other infections, no clinical features distinguish these cases from those caused by other members of the Enterobacteriaceae.

### Intestinal Infections

ETEC and EPEC diarrhea is watery

EHEC diarrhea is bloody

Infections caused by all of the *E. coli* virulence types usually begin with a mild watery diarrhea starting 2 to 4 days after ingestion of an infectious dose. In most instances, the duration of diarrhea is limited to a few days, with the exception of EAEC diarrhea, which can last for weeks. With ETEC and EPEC, the diarrhea remains watery, but with EIEC and EHEC, a dysenteric illness follows. Some EPEC cases may also become chronic. EHEC disease begins like the others but often also includes vomiting. In 90% of cases this is followed in 1 to 2 days by intense abdominal pain and bloody diarrhea, but fever is not prominent. Some EHEC cases develop into a dysentery that is less severe than that seen in shigellosis. Colonoscopy reveals edema, hemorrhage, and pseudomembrane formation. Resolution usually takes place over a 3- to 10-day period, with few residual effects on the bowel mucosa.

HUS begins as oliguria and may progress to renal failure

HUS develops as a complication in about 10% of cases of EHEC hemorrhagic colitis, primarily in children under 10 years of age. The disease begins with oliguria, edema, and pallor, progressing to the triad of microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. The systemic effects are often life-threatening, requiring transfusion and hemodialysis for survival. The mortality rate is 5%, and as many as 30% of those individuals who survive suffer sequelae such as renal impairment or hypertension.

## DIAGNOSIS

Like the rest of the Enterobacteriaceae, *E. coli* is readily isolated in culture. For the diagnosis of intestinal disease, separating the virulent types discussed above from the numerous other *E. coli* strains commonly found in stool presents a special problem. A myriad of immunoassay and nucleic acid methods have been described that are able to detect the toxins and genes associated with virulence. These methods work but are still too expensive to be practical, especially in the developing countries where ETEC, EIEC, EPEC, and EAEC are prevalent. A screening test for EHEC takes advantage of the observation that the O157:H7 serotype typically fails to ferment sorbitol. Incorporating sorbitol in place of lactose in MacConkey agar provides an indicator medium from which suspect (colorless) colonies can be selected and then confirmed with O157 antisera. This procedure has become routine in areas where EHEC is endemic.

Methods to detect virulence factors are expensive

Sorbitol agar screens for O157:H7

## TREATMENT

Because most *E. coli* diarrheas are mild and self-limiting, treatment is usually not an issue. When it is, rehydration and supportive measures are the mainstays of therapy, regardless of the causative agent. In the case of EHEC with hemorrhagic colitis and HUS, heroic supportive measures such as hemodialysis or hemapheresis may be required. Treatment with trimethoprim/sulfamethoxazole (TMP-SMX) or quinolones reduces the duration of diarrhea in ETEC, EIEC, and EPEC infection, but neither the course of hemorrhagic colitis nor the risk of HUS are altered by antimicrobial therapy. Because the risk of HUS may be increased by antimicrobial treatments, many physicians feel that treatment is not indicated. Antimotility agents are not helpful and are contraindicated when EIEC or EHEC might be the etiologic agent.

Antimicrobics may help all but EHEC

## PREVENTION

Traveler's diarrhea is usually little more than an inconvenience. Because the infecting dose is high, the incidence of the disease can be greatly reduced by eating only cooked foods and peeled fruits, and drinking hot or carbonated beverages. Avoiding uncertain water, ice, salads, and raw vegetables is a wise precaution when traveling in developing countries. High-priced hotel accommodations have no protective effect. Chemoprophylaxis against traveler's diarrhea is not routinely recommended. TMP-SMX or ciprofloxacin have been recommended for a short-term (<2 weeks) for those at high risk for disease resulting from such chronic conditions as achlorhydria, gastric resection, prolonged use of $H_2$ blockers or antacids, and underlying immunosuppressive diseases.

Avoid uncooked foods

Chemoprophylaxis works for defined periods

These public health measures apply equally to EHEC, but here prevention is more difficult because the infecting dose is so low. Cooking hamburgers all the way through is sensible, but no one is recommending abstinence from salads when at home. Recent US recommendations for the irradiation of meats and the extension of pasteurization requirements to fruit juices are largely designed to stem the spread of EHEC.

Rare hamburgers carry risk for EHEC

# SHIGELLA

## BACTERIOLOGY

*Shigella* species are closely related to *E. coli*. Most fail to produce gas when fermenting glucose and do not ferment lactose. Their antigenic makeup has been characterized in a manner similar to *E. coli* with the exception that they lack flagella and thus H antigens.

All *Shigella* species are nonmotile. The genus is divided into four species which are defined by biochemical reactions and specific O antigens organized into serogroups. The species are *Shigella dysenteriae* (serogroup A), *Shigella flexneri* (serogroup B), *Shigella boydii* (serogroup C), and *Shigella sonnei* (serogroup D). All but *S. sonnei* are further subdivided into a total of 38 individual O antigen serotypes specified by numbers. *Shigella* is the prototype invasive bacterial pathogen. All species are able to invade and multiply inside a wide variety of epithelial cells, including their natural target, the enterocyte. *S. dysenteriae* type A1 (Shiga bacillus), the species that was first discovered, is the most potent producer of Shiga toxin. Other *Shigella* species produce various molecular forms of Shiga toxin.

O antigens and biochemicals define four species

Invasiveness and Shiga toxin production are virulence factors

## SHIGELLOSIS

CLINICAL CAPSULE

*Shigella* is the classic cause of dysentery, which is typically spread person-to-person under poor sanitary conditions. The illness begins as a watery diarrhea but evolves into an intense colitis with frequent small-volume stools that contain blood and pus. Despite the invasive properties of the causal organism, the infection usually does not spread outside the intestinal tract.

### EPIDEMIOLOGY

Shigellosis is a strictly human disease with no animal reservoirs. In the United States, the number of reported cases has remained in the range of 8 to 12 cases per 100,000 population for over 30 years. Worldwide, it is consistently one of the most common causes of infectious diarrhea in both developed and developing countries, and it is estimated to cause 600,000 deaths per year. The organisms can be readily transmitted by the fecal–oral route through person-to-person contact or by contamination of food or water. This mode of spread is efficient; the infecting dose is less than 200 organisms in volunteer studies. The secondary attack rates among family members are as high as 40%.

Low infecting dose facilitates fecal–oral spread

Strictly human disease

The incidence and spread of shigellosis is directly related to personal and community sanitary practices. In developed countries, it is largely a pediatric disease. In countries where the sanitary infrastructure is inadequate and in institutions plagued by crowding and poor hygienic conditions the disease may be more widespread. Wartime and natural disasters create similar circumstances. The most common species are *S. sonnei* and *S. flexneri,* with *S. dysenteriae* largely limited to underdeveloped tropical areas. *S. dysenteriae,* type 1 produces the most severe disease, historically known as "bacillary dysentery." This condition has slowed the march of many an army; it was the leading cause of death in the notorious Andersonville prison camp during the American Civil War.

Personal and community sanitary practices determine incidence

Wars and disasters create outbreaks

### PATHOGENESIS

*Shigella,* unlike *Vibrio cholerae* and most *Salmonella* species, is acid-resistant and survives passage through the stomach to reach the intestine. Once there, the fundamental pathogenic event is invasion of the human colonic mucosa. This triggers an intense acute inflammatory response with mucosal ulceration and abscess formation. Invasion and spread is a multistep process (Fig 21–5), which is the same in *Shigella* and EIEC.

Bacteria pass stomach acid and invade colon

*Shigella* initially crosses the mucosal membrane by entering the follicle-associated M cells of the intestine, which lack the highly organized brush borders of absorptive enterocytes. The *Shigella* adhere selectively to M cells and can transcytose through them into the underlying collection of phagocytic cells (Fig 21–6). Bacteria inside M cells and phagocytic macrophages are able to cause their demise by activating normal programmed cell death (apoptosis). Bacteria released from the M cell contact the basolateral side of

M cells are transcytosed

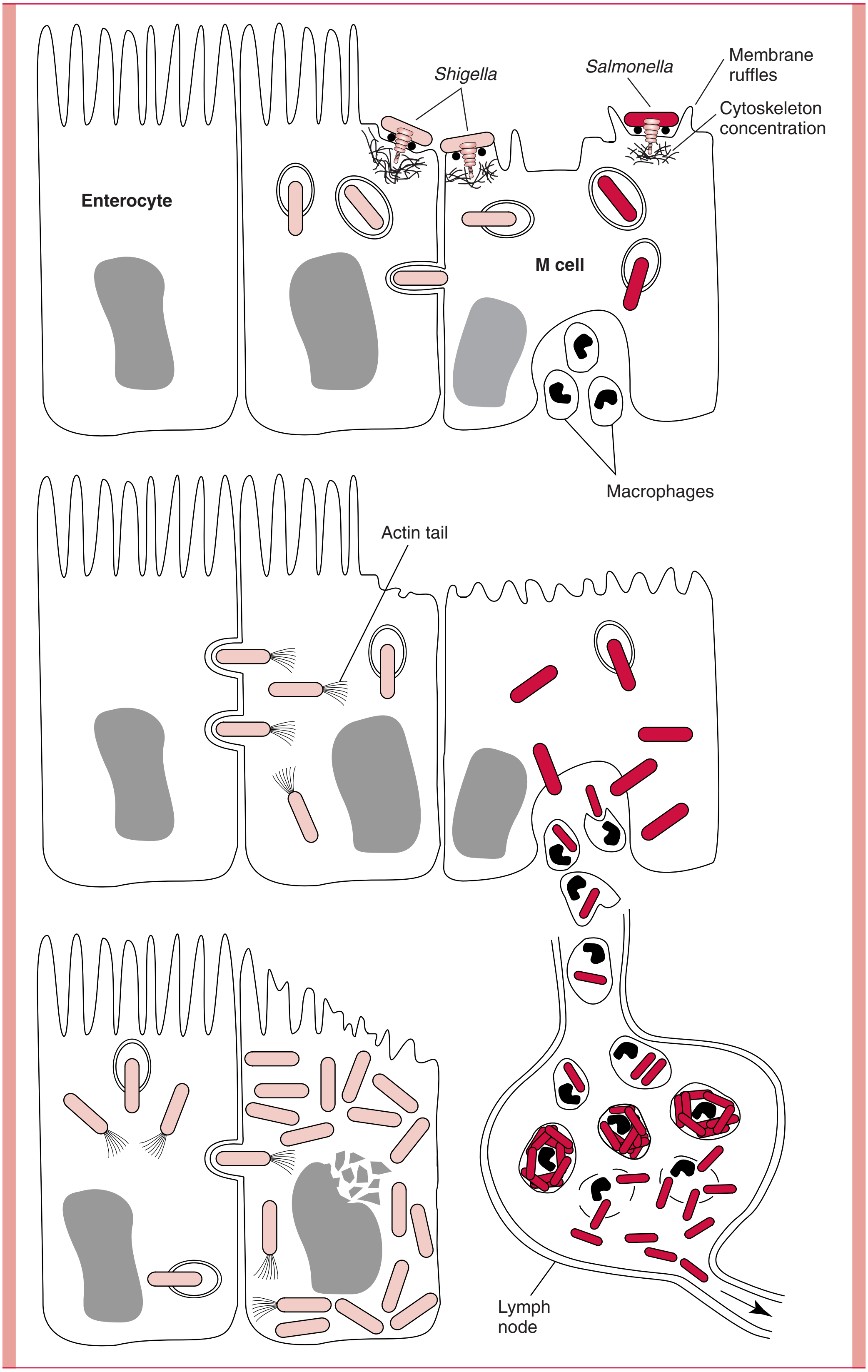


FIGURE 21–5
Invasion by *Shigella flexneri* and *Salmonella* serotype Typhi. The *Shigella* and *Salmonella* are shown invading the intestinal M cells but taking different paths after escaping the endocytotic vacuole. The *Shigella* multiplies in the cell and propels itself through the cytoplasm to invade adjacent cells, and the *Salmonella* passes through the cell to the submucosa, where it is taken up by macrophages. Serotype Typhi is able to multiply in the macrophages in the lymph node and other reticuloendothelial sites. Both organisms induce apoptosis in their host cells. In the case of *Shigella,* this produces a mucosal ulcer; in the case of Typhi, it leads to seeding of the bloodstream and typhoid fever.

FIGURE 21–6
*Salmonella* entering an M cell. Two organisms (arrows) are seen attaching to the M cell surface. Note the contrast with the flanking enterocytes and the macrophage just below the M cell.

Injected Ipa proteins direct stages of enterocyte invasion

Cytoskeleton accumulation leads to endocytosis

enterocytes and initiate a multistep invasion process mediated by a set of **invasion plasmid antigens** (IpaA, IpaB, IpaC). On contact with the enterocyte, these antigens are injected by a contact secretion system and each has its individual action. These include cell attachment, cytoskeleton reorganization, actin polymerization, and induction of apoptosis. Rather than create A/E lesions as with EPEC and EHEC, this cytoskeleton modification process involves accumulation of filamentous actin underneath the host cell cytoplasmic membrane, inducing engulfment and internalization of the bacterium into the host cell by endocytosis.

Polymerization of cytoskeletal actin propels bacteria

Adjacent enterocytes are invaded directly

Double-membrane lysis restarts process

*Shigella* brought into cells are highly adapted to the intracellular environment and make unique use of it to continue the infection. Although initially the bacteria are surrounded by a phagocytic vacuole, they escape within 15 minutes and enter the cytoplasmic compartment of the host cell. Almost immediately, they orient in parallel with the filaments of the actin cytoskeleton of the cell and initiate a process in which they control polymerization of the monomers that make up the actin fibrils. This process creates an actin "tail" at one end of the microbe, which appears to propel it through the cytoplasm like a comet. This exploitation of the cytoskeletal apparatus allows nonmotile *Shigella* to not only replicate in the cell but to move efficiently through it. Eventually, the bacteria encounter the host cell membrane, much of which is adjacent to the neighboring enterocytes. At this point some *Shigella* rebound, but others push the membrane as much as 20 $\mu$m into the adjacent cell. This invasion of the neighboring enterocyte forms fingerlike projections, which eventually pinch off, placing the bacterium within a new cell but surrounded by double membrane. The organisms then lyse both membranes and are released into the cytoplasm, free to begin the cycle anew.

Enterocyte invasion creates ulcers

Diarrhea + WBCs + RBCs = dysentery

The cell-by-cell extension of this process radially creates focal ulcers in the mucosa, particularly in the colon. The ulcers add a hemorrhagic component and allow *Shigella* to reach the lamina propria, where they evoke an intense acute inflammatory response. Extension of the infection beyond the lamina is unusual in healthy individuals. The diarrhea created by this process is almost purely inflammatory, consisting of small volume stools containing WBCs, RBCs, bacteria, and little else. This is classic dysentery.

Some *Shigella* produce Shiga toxin, which is not essential for disease, but does contribute to the severity of the illness. The original and most potent producer of Shiga toxin, *S. dysenteriae* type 1, is the only *Shigella* with a significant mortality rate among previously healthy individuals. This is probably due to systemic effects of the toxin, which can be the same as described above for the EHEC, including HUS. Enterotoxins have also been described that may be the basis of the watery diarrhea sometimes observed in the early phases.

Shiga toxin increases severity of disease

All virulent *Shigella* and EIEC carry a very large plasmid that has several genes essential for the attachment and entry process, including the Ipa genes. The characteristics of *Shigella* entry and interaction with cellular elements are very similar to those observed with *Listeria monocytogenes* (see Chapter 18), which is Gram-positive, motile, and prefers livestock to humans. Finding that such dissimilar bacteria use such similar tactics to infect their preferred host suggests that this represents a common thread in the selective pressures for a microbe to become a "successful" enteric pathogen.

Large plasmid containing Ipa genes is required for virulence

## IMMUNITY

*Shigella* infection produces relatively short-lived immunity to reinfection with homologous serogroups. There is no consensus on the mechanisms involved.

Immunity is brief

# SHIGELLOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

*Shigella* organisms cause an acute inflammatory colitis and bloody diarrhea, which in the most characteristic state presents as a dysentery syndrome—a clinical triad consisting of cramps, painful straining to pass stools (tenesmus), and a frequent, small-volume, bloody, mucoid discharge. However, most clinical shigellosis due to *S. sonnei* in the United States is a watery diarrhea that is often indistinguishable from that of other bacterial or viral diarrheal illness. The disease usually begins with fever and systemic manifestations of malaise, anorexia, and sometimes myalgia. These nondescript symptoms are followed by the onset of watery diarrhea containing the large numbers of leukocytes detectable by light microscopy. The diarrhea may turn bloody with or without the other classical signs of dysentery. The manifestations may be more severe when *S. flexneri,* the species that predominates in the developing world, is involved and most severe with *S. dysenteriae* type 1 (Shiga bacillus). Although the vast majority of shigellosis cases resolve spontaneously after 2 to 5 days, the mortality in Shiga epidemics in Asia, Latin America, and Africa has been as high as 20%.

Watery diarrhea is followed by fever, bloody mucoid stools, and cramping

Mortality significant with *S. dysenteriae* type 1

Most infections are self-limiting

## DIAGNOSIS

All *Shigella* species are readily isolated using selective media (e.g. Hektoen enteric agar) which are part of the routine stool culture in clinical laboratories. These media contain chemical additives empirically shown to inhibit facultative flora (eg, *E. coli, Klebsiella*), with relatively little effect on *Shigella* (or *Salmonella*). They also contain indicator systems which utilize typical biochemical reactions to mark suspect *Shigella* colonies among the other flora. Isolates are identified with further biochemical tests. Slide agglutination tests using O group specific antisera (A, B, C, D) confirm both the species and the *Shigella* genus.

Selective media are routinely used

O antigens confirm species

## TREATMENT

Several antimicrobics have proved effective in the treatment of shigellosis. Because the disease is usually self-limiting, the beneficial effect of treatment is in shortening the illness and the period of excretion of organisms. Ampicillin was once the treatment of

Treatment may shorten illness and period of excretion

Ampicillin resistance is common

choice, but resistance rates of 5 to 50% have caused a shift to TMP-SMX in many areas. In recent years, quinolones and third-generation cephalosporins have been used in the face of resistance to other agents. Antispasmodic agents may aggravate the condition and are contraindicated in shigellosis and other invasive diarrheas.

## PREVENTION

Sanitation, insect control, handwashing, and cooking block transmission

Live attenuated vaccines are under investigation

Standard sanitation practices such as sewage disposal and water chlorination are important in preventing the spread of shigellosis. In certain circumstances, insect control may also be important, because flies can serve as passive vectors when open sewage is present. Good individual sanitary practices, such as handwashing and proper cooking of food, are highly protective. Parenteral vaccines have proved disappointing, and current efforts are directed toward finding orally administered live vaccines that can stimulate mucosal IgA. Many strains, including attenuated *Shigella* mutants, *E. coli–Shigella* genetic hybrids, and *E. coli* with genes for some (but not all) the invasive (Ipa) proteins, are vaccine candidates. The general idea is to find a strain that will go through enough of the multistage process (see Pathogenesis) to stimulate an immune response but stop short of full penetration and spread.

# SALMONELLA

## BACTERIOLOGY

Complexity of O, K, and H antigens leads to many serotypes

Historic names persist as serotypes of *S. enterica*

More than any other genus, *Salmonella* has been a favorite of those who love to subdivide and apply names to biologic systems. At one time, there were over 2000 names for various members of this genus, often reflecting colorful aspects of place or circumstances of the original isolation (eg, *S. budapest, S. seminole, S. tamale, S. oysterbeds*). This has now been reduced to a single species, *Salmonella enterica,* with the previous species names relegated to the status of serotypes. All of this is made particularly robust by the fact that, in addition to a large number of the LPS O and some capsular K antigens, the flagellar H antigens of most *Salmonella* undergo phase variation. This adds the prospect of two sets of H antigens to the already complex system. As in *Shigella,* the specific O antigens are organized into serogroups (eg, A, B, . . . K, and so on) to which the two H and K (if present) antigen designations are appended to achieve the full antigenic formula. It is not difficult to understand why microbiologists, when confronted with a salmonella with the antigenic formula O:group B [1,4,12] H:I;1,2, still prefer to call it *Salmonella typhimurium.* The proper name for this organism is *Salmonella enterica* serotype Typhimurium, but indulging in the convenience of elevating the serotype to species status is still common.

*Salmonella* species vary in preferred host

*S. typhi* infects only humans

Another feature distinguishing *Salmonella* serotypes is their host range. Some are highly adapted to particular mammals or amphibians, and others infect a broad range of hosts. Of interest for medical microbiology are those that infect humans and other animals and those strictly adapted to humans and higher primates. *S. enterica* serotype Typhimurium is the prototype for the former and *S. enterica* serotype Typhi for the latter. In the following discussions, Typhi will be used for the strictly human species that produce enteric (typhoid) fever. Unless otherwise specified, *S. enterica* will be used for the serotypes that are able to infect animals or humans and typically cause gastroenteritis in the latter.

Pili and flagella are functional

Salmonellae possess multiple types of pili, one of which is morphologically and functionally similar to the *E. coli* type 1 pili, which bind D-mannose receptors on various eukaryotic cell types. Most strains are motile through the action of their flagella.

## SALMONELLA GASTROENTERITIS (*S. enterica*)

CLINICAL CAPSULE

The typical example of *Salmonella* "food poisoning" is the community picnic or bazaar, where volunteers prepare poultry, salads, and other potential culture media to be eaten later in the day. Because the refrigerators are filled with beer and soda, the food is left out in covered pans. A near physiologic incubation temperature is provided by the still-warm contents and the afternoon sun. This allows the organisms to enter logarithmic growth during the softball game. The bacteria usually produce no noticeable change in the food. One to two days after the feast, a significant portion of the revelers develop abdominal pain, nausea, vomiting, and diarrhea lasting for 3 or 4 days. An investigation points to a particular food such as potato salad or turkey dressing, which is found to have a correlation with both attack rate and severity of illness.

## EPIDEMIOLOGY

*S. enterica* gastroenteritis is predominantly a disease of industrialized societies and improper food handling, which allows the transmission from the animal reservoir to humans. The infecting dose delivered in contaminated food is higher than with *Shigella*. Ingestion of 1000 or more *Salmonella* bacilli is required to cause illness, making direct human-to-human transmission difficult. Achlorhydric individuals or those taking antacids can be infected with considerably smaller inocula. Consistently, *Salmonella* are a leading cause of food-borne intestinal infection under conditions similar to those described in the above capsule.

Infecting dose is higher than *Shigella*

Poultry products, including eggs infected transovarially, are most often implicated as the vehicle of infection of *Salmonella* gastroenteritis. Food preparation practices that allow achievement of an infecting dose by growth of the bacteria in the food prior to ingestion are most commonly involved. The incidence in the United States is approximately double that of *Shigella,* with 40,000 to 50,000 reported cases per year. This is believed to reflect only about 1 to 5% of the actual infections. The number of cases varies seasonally, with peak incidence in summer and fall.

Poultry products are common source

The highest rates of infection are in children less than 5 years old, persons aged 20 to 30, and those older than 70. If one household member becomes infected, the probability that another will become infected approaches 60%. Nearly one third of all *Salmonella* epidemics occur in nursing homes, hospitals, mental health facilities, and other institutions. A recent increase in the popularity of raw milk has been associated with outbreaks of *Salmonella* (and *Campylobacter*) infection. Exotic pets such as turtles have also been the source of infection. Humans can also be the source of disease. Fully 5% of patients recovering from gastroenteritis still shed the organisms 20 weeks later. Chronic carriers who are food handlers are an important reservoir in the epidemiology of food-borne disease.

Outbreaks in institutions are common

Human carriers can be a source

In recent years, the epidemiology of salmonellosis has changed, and the number of multistate outbreaks has increased, often through the contamination of foodstuffs during large-scale production at a single plant. Efficient interstate and international distribution systems that deliver large amounts of the contaminated food over a wide area facilitate spread. Under these conditions, an attack rate as low as 0.5% can still produce many infections, because of the large number of individuals at risk. It is of concern that relatively small numbers of cases sprinkled over a massive area will be missed by local surveillance systems crippled by budgetary cutbacks.

Modern delivery systems can spread disease efficiently

## PATHOGENESIS

Ingested *S. enterica* cells that pass the stomach acid and swim through the intestinal mucous layer eventually reach the enterocytes and M cells of the large and small bowel. Adherence is probably mediated by pili, but on initial contact of bacteria with M cells, the stimulation of membrane "ruffles" dramatically alters the normal architecture within minutes (Fig 21–7).

Adherence triggers surface ruffles

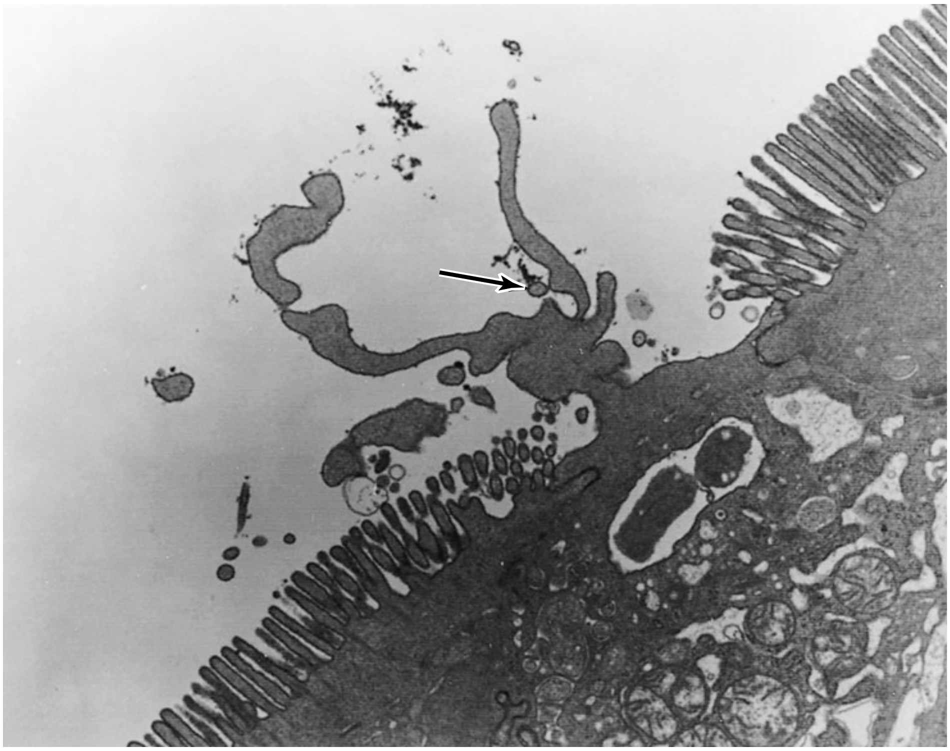

FIGURE 21–7
*Salmonella* membrane ruffles. These extensions of the plasma membrane are stimulated by the *Salmonella* (arrow) and are related to internalizing the bacteria.

These "ruffles" are specialized mammalian plasma membrane sites of filamentous actin cytoskeletal rearrangement normally induced by physiologic molecules such as growth factors. In the case of *Salmonella,* they are stimulated by one or more of a family of more than 12 proteins whose genes (invA, invB, spaP, spaQ) are located in at least two PAIs inserted into the *Salmonella* genome. The virulence factors coded by the genes in the PAIs are either components of the apparatus of a contact secretion system or the effector proteins it injects.

Secretion system genes are in PAIs

The "ruffles" seem to engulf the organism in an endocytotic vacuole and allow it to transcytose from the apical surface to the basolateral membrane. Once through the cell, the organisms enter the lamina propria, where they induce a profound inflammatory response. When taken up by macrophages, they are able to persist by inducing apoptosis of the phagocyte. Genes for macrophage survival are located in a second PAI. This process contrasts with *Shigella,* which escapes the endocytotic vacuole (and double vacuole) and prefers to invade adjacent enterocytes rather than move through to the submucosa.

"Ruffles" create transcytosed vacuoles

Macrophage apoptosis aids survival

Although some enterotoxins have been described in *Salmonella,* their role in diarrhea is unclear. The best estimate is that the invasion and transcytosis of enterocytes together with the associated increased vascular permeability and inflammatory response are enough to account for the diarrhea. The release of prostaglandins and chemotactic factors may trigger inflammation and biochemical changes in enterocytes. Although the process remains localized to the mucosa and submucosa with most *S. enterica* strains, some invade more deeply, reaching the bloodstream and distant organs. Some serotypes (eg, Choleraesuis) even invade so rapidly that they produce minimal diarrhea and are isolated more frequently from the blood than stool.

Enterotoxin role is unclear

Invasion and inflammation cause diarrhea

## IMMUNITY

Evidence that both humoral and cell-mediated immune responses are stimulated by infection with *S. enterica* is ample. How these processes relate to immunity and control of the bacterial infection is largely unknown.

Immune mechanisms unclear

## ENTERIC (TYPHOID) FEVER (*Salmonella serotype Typhi*)

CLINICAL CAPSULE

Typhoid is the fever of the phrase "she died of a fever," as in Victorian novels or the street ditty of sweet Molly Malone. Typhoid fever has a slow, insidious onset and if untreated, lasts for weeks. It ends either by a gradual resolution or in death due to complications (eg, rupture of the intestine or spleen). Family members may note only the extended fever, although physicians may observe a subtle rash or feel an enlarged spleen. Diarrhea may occur once or twice during the course but is not a consistent feature.

### EPIDEMIOLOGY

Typhoid is a strictly human disease. Chronic carriers of serotype Typhi are the primary reservoir. Some patients become chronic carriers for years (hence the famous "typhoid Mary" Mallon), typically because of chronic infection of the gallbladder and the biliary tract when stones are present. All cases should be traced back to their human source. If a patient with typhoid has not traveled to an endemic area, the source must be a visitor or someone else who prepared food. The pathogen can be transmitted in the water supply in developing endemic areas or where defects in any system allow sewage from carriers to contaminate drinking water. Transmission is by the fecal–oral route. The infecting dose of $10^5$ to $10^6$ bacteria is intermediate between *Shigella* and most *S. enterica* and decreases in the presence of the capsular Vi antigen.

Cases are traceable to a human source

Fecal–oral transmission requires moderate dose

Typhoid fever is still an important cause of morbidity and mortality worldwide. In the United States and most other industrialized nations, it is mostly seen in travelers to endemic areas such as Latin America, Asia, and India. Visitors from these areas who are carriers are often the source of isolated cases. The decline in disease in industrialized nations largely reflects the availability of clean water supplies and improved disposal of fecal waste.

Prevalence is linked to sanitary infrastructure

### PATHOGENESIS

As there is no animal model for the strictly human Typhi, the details of the cellular events are inferred from studies of Typhimurium, which in mice produces a disease similar to typhoid (thus the name). The invasion and killing of intestinal M cells and macrophages are presumed to follow the same pattern as *S. enterica*. Two differences are the surface polysaccharide Vi antigen and the extended multiplication of Typhi in macrophages. In the submucosa, the Vi antigen retards polymorphonuclear neutrophil (PMN) phagocytosis by interfering with complement deposition in a manner similar to other bacterial surface polysaccharides. This may favor uptake by macrophages where at least some Typhi cells establish a privileged niche. Like other serotypes of *Salmonella,* the typhoid bacteria remain within a membrane-bound vacuole and replicate, leading in many cases to macrophage death.

Typhi invades M cells and macrophages

Vi polysaccharide limits PMN phagocytosis

The primary difference between Typhi and the other serotypes is its prolonged intracellular survival in macrophages. This is due to the organism's ability to inhibit the oxidative metabolic burst and continue to multiply. As the bacteria proliferate in macrophages, they are carried through the lymphatic circulation to the mesenteric nodes, spleen, liver and bone marrow, all elements of the reticuloendothelial system (RES). At the RES sites, Typhi continues to multiply, infecting new host macrophages, but eventually the bacteria begin to spill into the bloodstream. This seeding of Gram-negative bacteria and their LPS endotoxin starts the fever, which increases and persists with the continuing bacteremia, sometimes resulting in infection of the urinary tract and other organs. Spread to the biliary tree leads to reinfection of the bowel. This cycle beginning and ending in the small intestine takes approximately 2 weeks to complete.

Inhibition of oxidative burst prolongs macrophage survival

RES sites seed the bloodstream and other organs

Endotoxin produces the fever

## IMMUNITY

Immunity follows natural infection

The immune response to enteric fever is both humoral and cell mediated. In nonfatal cases, humoral antibody and activated macrophages eventually subdue the untreated infection over a period of about 3 weeks. Reinfection is rare unless the course was shortened by early administration of antimicrobics. Which antigens stimulate this immunity is not clearly understood. The Vi antigen is usually credited, but various surface proteins are also candidates.

# SALMONELLOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

*S. enterica* = gastroenteritis

Typhi = enteric fever

The clinical patterns of salmonellosis can be divided into gastroenteritis, bacteremia with and without focal extraintestinal infection, enteric fever, and the asymptomatic carrier state. Any *Salmonella* serotype can probably cause any of these clinical manifestations under appropriate conditions, but in practice the *S. enterica* serotypes are associated primarily with gastroenteritis. Typhi and a few related serotypes (Paratyphi) cause enteric fever.

### Gastroenteritis

Diarrhea, vomiting, and cramps are common

Typically, the episode begins 24 to 48 hours after ingestion, with nausea and vomiting followed by, or concomitant with, abdominal cramps and diarrhea. Diarrhea persists as the predominant symptom for 3 to 4 days and usually resolves spontaneously within 7 days. Fever (39°C) is present in about 50% of the patients. The spectrum of disease ranges from a few loose stools to a severe dysentery-like syndrome.

### Bacteremia and Metastatic Infection

Bacteremia is most common and severe in immunocompromised

Metastatic sites linked to previous injury particularly sickle-cell

The acute gastroenteritis caused by *S. enterica* can be associated with transient or persistent bacteremia. Frank sepsis is uncommon, except in those with a compromised cell-mediated immune system. *Salmonella* infection in patients with acquired immunodeficiency syndrome (AIDS) is common and often severe. Bacteremia occurs in 70% of these patients and can cause septic shock and death. Despite adequate antimicrobial coverage, relapses are frequent. Patients with lymphoproliferative disease, perhaps owing to T-cell defects similar to those in patients with AIDS, are also highly susceptible to disseminated salmonellosis. Metastatic spread by salmonellae is a significant risk when bacteremia occurs. These organisms have a unique ability to colonize sites of preexisting structural abnormality including atherosclerotic plaques, sites of malignancy, and the meninges (especially in infants). *Salmonella* infection of the bone typically involves the long bones; in particular, sites of trauma, sickle cell injury, and skeletal prosthesis are at risk.

### Enteric Fever

Slowly increasing fever lasts for weeks

Diarrhea is intermittent or absent

Enteric fever is a multiorgan system *Salmonella* infection characterized by prolonged fever, sustained bacteremia, and profound involvement of the RES, particularly the mesenteric lymph nodes, liver, and spleen. The manifestations of typhoid (Fig 21–8) have been well documented in human volunteer studies conducted during vaccine trials. The mean incubation period is 13 days, and the first sign of disease is fever associated with a headache. The fever rises in a stepwise fashion over the next 72 hours. A relatively slow pulse is characteristic and out of phase with the elevated temperature. In untreated patients, the elevated temperature persists for weeks. A faint rash (rose spots) appears during the first few days on the abdomen and chest. Few in number, these spots are readily overlooked, especially in dark-skinned individuals. Many patients are constipated, although perhaps one third of patients have a mild diarrhea. As the untreated disease progresses, an increasing number of patients complain of diarrhea.

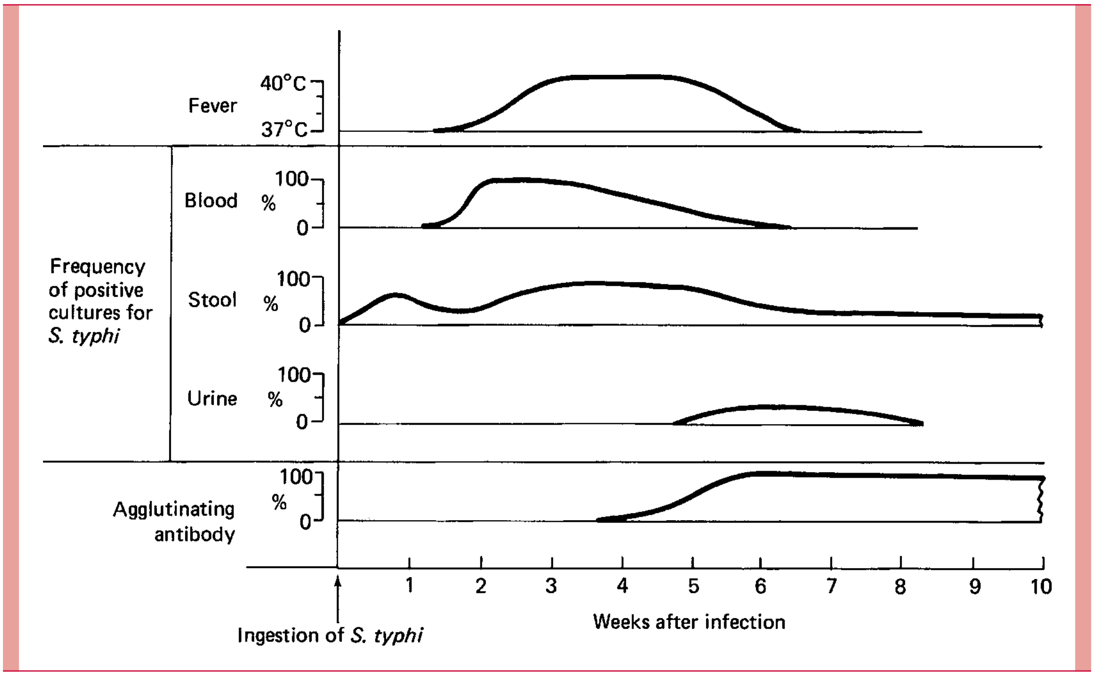


FIGURE 21–8
Natural history of enteric (typhoid) fever. The course of disease without antimicrobial therapy. Fever chart shows time course for typical patient. Culture and agglutinating antibody show timing and probability of positive results in a group of typhoid fever patients.

Obviously, chronic infection of the bloodstream is a serious disease, and the effects of endotoxin can lead to myocarditis, encephalopathy, or intravascular coagulation. Moreover, the persistent bacteremia can lead to infection at other sites. Of particular importance is the biliary tree, with reinfection of the intestinal tract and diarrhea late in the disease. UTI and metastatic lesions in bone, joint, liver, and meninges may also occur. However, the most important complication of typhoid fever is hemorrhage from perforations through the wall of the terminal ileum at the site of necrotic Peyer's patches or in the proximal colon. These occur in patients whose disease has been progressing for 2 weeks or more.

Biliary tree infection reseeds intestine

Urinary tract, bone, and joints are metastatic sites

## DIAGNOSIS

Culture of *Salmonella* from the blood or feces is the primary diagnostic method. Early in the course of enteric fever, blood is far more likely to give a positive culture result than culture from any other site. The media used for stool culture are the same as those used for *Shigella.* Failure to ferment lactose and the production of hydrogen sulfides from sulfur-containing amino acids are characteristic features used to identify suspect colonies on the selective isolation media. Characteristics of biochemical tests are used to identify the genus, and O serogroup antisera are available in larger laboratories for confirmation. Typhi has a pattern of biochemical reactions which are sufficient to characterize it without reference to its serogroup (D). All isolates should be referred to public health laboratories for confirmation and epidemiologic tracing. Serologic tests are no longer used for diagnosis.

Stool and blood culture are routine

Typhi has characteristic features

## TREATMENT

The primary therapeutic approach to *Salmonella* gastroenteritis is fluid and electrolyte replacement and the control of nausea and vomiting. Antibiotic therapy is usually not appropriate because it has a tendency to increase the duration and frequency of the carrier state. When used to eradicate the carrier state it meets with erratic success and usually fails in the presence of coexisting biliary tract disease. In patients with underlying risk

Antimicrobics are of limited use in gastroenteritis factors, antimicrobial treatment is used as a prophylactic measure aimed at preventing systemic spread.

Typhoid responds to chemotherapy but resistance is common

Chloramphenicol was the first antibiotic to be used to treat typhoid in 1948, and it reduced the mortality from 20% to less than 2%. Although resistance has developed, it is still a preferred drug in developing countries because it is inexpensive. Ampicillin and trimethoprim–sulfonamide have been used successfully to treat infections caused by chloramphenicol-resistant strains. Newer cephalosporins (ceftriaxone) and quinolones (ciprofloxacin, norfloxacin) are also effective. With proper antimicrobial therapy, patients feel better in 24 to 48 hours, their temperature returns to normal in 3 to 5 days, and they are generally well in 10 to 14 days.

## PREVENTION

Typhoid vaccines are only moderately effective

Sanitation and public health measures can eliminate Typhi

Typhoid vaccines have been available since before the turn of the century. An intramuscular killed whole bacterial vaccine was widely used by the military and in travelers but gave poor protection against exposure to large doses of organisms. Recently, a live oral vaccine containing an attenuated Typhi strain has been licensed. Probably as effective as the injectable vaccine, it protects as many as 70% of children in endemic areas. No human vaccine is available for the other *Salmonella* serotypes. When all is said and done, the provision of clean water supplies and the treatment of carriers will lead to the disappearance of typhoid. The importance of carriers and sanitation was emphasized by a 1973 typhoid outbreak among migrant workers in Florida. The source was traced to leakage of sewage into the water supply, failure of chlorination, and a chronic carrier.

# *YERSINIA*

## BACTERIOLOGY

Coccobacillary and grow at variable temperatures

Human pathogens are linked to animals

Morphologically, *Yersinia* tends to be coccobacillary and to retain staining at the ends of the cells (bipolar staining). In general, growth and metabolic characteristics are the same as those of other Enterobacteriaceae, although some strains grow more slowly or have optimal growth temperatures below 37°C. The genus includes 11 species, of which *Yersinia pestis, Yersinia pseudotuberculosis,* and *Yersinia enterocolitica* are the important pathogens for humans. *Yersinia pestis* is antigenically homogenous, but *Y. pseudotuberculosis* and *Y. enterocolitica* have multiple O and H antigens. *Yersinia* are primarily animal pathogens, with occasional transmission to humans through direct or indirect contact. *Y. pestis,* the cause of plague, is discussed primarily in Chapter 32, although features of its pathogenesis common to other *Yersinia* are included in this discussion.

## YERSINIA DISEASES (*Y. pseudotuberculosis* and *Y. enterocolitica*)

CLINICAL CAPSULE

Enteropathogenic species of *Yersinia* produce diseases associated with the gastrointestinal tract, ranging from simple gastroenteritis with diarrhea and vomiting to syndromes in which the primary features are abdominal pain and fever. *Yersinia* mesenteric adenitis can simulate acute appendicitis. *Y. enterocolitica* is one of the causes of the enteric fever syndrome.

## EPIDEMIOLOGY

In animals, *Y. pseudotuberculosis* causes pseudotuberculosis, a disease characterized by lesions ranging from local necrosis to granulomatous inflammation in the lymph nodes, spleen, and liver. The portal of entry for humans is the gastrointestinal tract, presumably by consumption of contaminated food or water. In most cases animals, including wild animals, are the most likely source of infection, but the exact mode of transmission is unknown. Geographic variation in the frequency of *Y. enterocolitica* infections is marked. The highest rates have been reported from some Scandinavian and other European countries, with much lower rates in the United Kingdom and the United States. Low isolation rates may be partially attributable to the difficulty of growing *Y. enterocolitica* from stool specimens.

Transmitted by ingestion from animal source

Geographic variation is great

## PATHOGENESIS

Enteropathogenic *Yersinia* entering the human host in contaminated food invade the M cells of the Peyer's patch. The invasive process and its effect on the host cell are driven by a large array of virulence factors that are deployed under complex genetic and environmental regulation. These proteins include **invasin,** which binds to integrins on the surface of host cells, and the ***Yersinia* outer membrane proteins (Yops),** which are the major effector proteins. The Yops are part of yet another contact secretion system that is deployed between the bacterial cell and host cell cytoplasm. When the Yops are injected into the host cell, they trigger cytotoxic events, including disruption of biochemical pathways (dephosphorylation, serine kinase), sensor functions, and the actin cytoskeleton.

Intestinal M cells are invaded

Secreted Yops disrupt cellular function

Some of the virulence factors produced by *Yersinia* are regulated in a system in which expression responds to either temperature or free calcium ($Ca^{2+}$) concentration. The physiologic temperature in a mammalian host is different from that in an insect or the environment, and the intracellular calcium concentration is markedly different from that of extracellular fluids. By sensing the environment, *Yersinia* is able to express or suppress virulence factors at different stages of the pathogenic process. The results seem timed to support the pathogenic strategy of *Yersinia,* which is to paralyze the phagocytic activity of defending macrophages and neutrophils and to nullify the host cellular immune response. The virulence determinants are encoded both on the bacterial chromosome and on a plasmid that contains genes for the secretion apparatus and the Yops. Another genetic component is a PAI, which is found only in the three pathogenic species and not the other *Yersinia*. The only known component of this PAI is an iron scavenging siderophore (yersiniabactin).

$Ca^{2+}$ and temperature regulate virulence factor expression

Plasmid and PAI contain virulence genes

The biological outcome of this extraordinary multifactorial process is the enhanced capacity of the pathogenic *Yersinia* to enter and replicate within the RES and to delay the cellular immune response. This leads to the formation of microabscesses and destruction of the cytoarchitecture of Peyer's patches and the mesenteric lymph nodes. The systemic symptoms seen with dissemination can largely be attributed to the effects of endotoxin.

Spread leads to microabscesses in lymph nodes

*Y. pestis* is a specialized variant closely related to *Y. pseudotuberculosis*. Instead of entering the intestinal tract *Y. pestis* reaches the dermal lymphatics by the bite of an infected flea. It has it own adhesin similar to that of invasin and two plasmids not found in the enteropathogenic *Yersinia*. Unique virulence factors for *Y. pestis* include a capsular protein antigen with antiphagocytic properties, a plasminogen activator protease that promotes adherence to basement membranes, and a fibrinolysin that may play a survival role in the flea.

*Y. pestis* has capsule, plasminogen activator, and fibrinolysin

# *YERSINIA* INFECTIONS: CLINICAL ASPECTS

Both *Y. enterocolitica* and *Y. pseudotuberculosis* cause acute mesenteric lymphadenitis, a syndrome involving fever and abdominal pain that often mimics acute appendicitis. *Y. enterocolitica* also produces a wider variety of manifestations. The most common of

Mesenteric lymphadenitis creates abdominal pain these is an enterocolitis, which usually occurs in children. It is characterized by fever, diarrhea, and abdominal pain. It also causes enteric fever, terminal ileitis, and a polyarthritic syndrome associated with its diarrheal manifestations. Few laboratories in the United States routinely screen stools for *Yersinia,* because yield has been low and good selective media are not available.

*Yersinia* are not routinely sought in stools

The role of antimicrobial therapy in the enteric *Yersinia* infections is uncertain, because they are usually self-limiting. *Y. pseudotuberculosis* is susceptible to ampicillin, cephalosporins, aminoglycosides, tetracyclines, and chloramphenicol, but *Y. enterocolitica* is usually resistant to penicillins and cephalosporins through the production of $\beta$-lactamases.

Antimicrobics have variable effect

## OTHER ENTEROBACTERIACEAE

All of the Enterobacteriaceae are capable of producing opportunistic infections of the type discussed above for *E. coli.* None are considered proven causes of enteric disease, although no doubt some will be in the future. The genera isolated in at least moderate frequency are discussed briefly below. There are many other less common species.

### *KLEBSIELLA*

The most distinctive bacteriologic features of the genus *Klebsiella* are the absence of motility and the presence of a polysaccharide capsule. This gives colonies a glistening, mucoid character and forms the basis of a serotyping system. Over 70 capsular types have been defined, including some that cross-react with those of other encapsulated pathogens, such as *Streptococcus pneumoniae* and *Haemophilus influenzae.* Limited studies suggest that the capsule interferes with complement activation in a way similar to the other encapsulated pathogens. Several types of pili are also present on the surface and probably aid in adherence to respiratory and urinary epithelium.

Polysaccharide capsule blocks complement deposition

*K. pneumoniae,* the most common species, is able to cause classic lobar pneumonia, a characteristic of other encapsulated bacteria. Most *Klebsiella* pneumonias are indistinguishable from those produced by other members of the Enterobacteriaceae. Of all the Enterobacteriaceae, *Klebsiella* species are now among the most resistant to antimicrobics.

Often are multiresistant

### *ENTEROBACTER*

*Enterobacter* species generally ferment lactose promptly and produce colonies similar to those of *Klebsiella,* although not as mucoid. A differential feature is motility by peritrichous flagella, which are generally present in *Enterobacter* species but uniformly absent in *Klebsiella*. *Enterobacter* species, which appear to be less virulent than *Klebsiella,* are usually found in mixed infections, in which their significance must be decided on clinical and epidemiologic grounds. Several hospital outbreaks traced to contaminated parenteral fluid solutions have implicated *Enterobacter* species. In addition to ampicillin, most isolates are resistant to first-generation cephalosporins, but may be susceptible to second- or third-generation cephalosporins; however, mutants derepressed for $\beta$-lactamase production occur at relatively high frequency and confer resistance to many cephalosporins.

Modest virulence but are linked to hospital contamination

### *SERRATIA*

*Serratia* strains ferment lactose slowly (3 to 4 days), if at all. Some produce distinctive brick-red colonies. Although less common, this genus produces the same range of opportunistic infections seen with the remainder of the Enterobacteriaceae. *Serratia* strains show consistent resistance to ampicillin and cephalothin, with the frequent addition of plasmid-determined resistance to many other antimicrobics, including the aminoglycosides.

Red pigment and multiresistance are characteristic

Sporadic infections and nosocomial outbreaks with multiresistant strains have often been difficult to control.

## *CITROBACTER*

The genus *Citrobacter,* although biochemically and serologically similar to *Salmonella,* is an uncommon cause of opportunistic infection; it does not cause enterocolitis or enteric fever. Like many other Enterobacteriaceae, *Citrobacter* strains may be present in the normal intestinal flora and cause opportunistic infections. Despite reports of association with diarrheal disease, present evidence does not indicate that *Citrobacter* should be considered an enteric pathogen. *C. freundii* has been associated with neonatal meningitis and brain abscess.

Opportunistic infection and brain abscess are uncommon

## *PROTEUS, PROVIDENCIA,* AND *MORGANELLA*

*Proteus, Morganella,* and *Providencia* are also opportunistic pathogens found with varying frequencies in the normal intestinal flora. *Proteus mirabilis,* the most commonly isolated member of the group, is one of the most susceptible of the Enterobacteriaceae to the penicillins; this characteristic includes moderate susceptibility to penicillin G. Other Proteeae are regularly resistant to ampicillin and the cephalosporins. *Proteus mirabilis* and *Proteus vulgaris* share the ability to swarm over the surface of media, rather than remaining confined to discrete colonies. This characteristic makes them readily recognizable in the laboratory—often with dismay, because the spreading growth covers other organisms in the culture and thus delays their isolation. *Proteus* and *Morganella* differ from other Enterobacteriaceae in the production of a very potent urease, which aids their rapid identification. It also leads to production of urinary stones and produces alkalinity and an ammoniac odor to the urine. *Providencia* species do not produce urease, are the least frequently isolated, and are generally the most resistant of the group to antimicrobics.

Swarming is a feature of some species

Urease production is linked to urinary stones

## ADDITIONAL READING

Darwin KH, Miller VI. Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. *Clin Microbiol Rev* 1999;12:405–428. A well-illustrated review of how *Salmonella* enters and moves through cells. It includes a discussion of the regulation of virulence factors.

Goosney DL, Knoechel DG, Finlay BB. Enteropathogenic *E. coli, Salmonella,* and *Shigella:* Masters of host cell cytoskeleton exploitation. *Emerging Infect Dis* 1999;5:214–223. A concise synopsis that finds similarities among the three major enteropathogenic members of the Enterobacteriaceae.

Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli. Clin Microbiol Rev* 1998;11:142–201. A very comprehensive review that includes pathogenesis and epidemiology as well as clinical aspects. Considerable attention is devoted to molecular diagnostics.

Schaechter M and The View From Here Group. *Escherichia coli* and *Salmonella* 2000: The View From Here. *Microbiol Mol Biol Rev* 2001;65:119–130. As suggested in the title, this is a broad, visionary view of these two pathogens, which extends beyond their medical importance.

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C H A P T E R 2 2

# *Vibrio, Campylobacter,* and *Helicobacter*

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This group includes *Vibrio cholerae,* the cause of cholera, one of the first proven infectious diseases, and two newcomers incriminated as pathogens in the past two decades (see Table 22–1). The peptic ulcer disease now known to be caused by *Helicobacter pylori* had been long accepted to be due to stress and disturbed gastric acid secretion. *Campylobacter jejuni* is one of the most common causes of diarrhea in virtually every country of the world. Cholera has undergone a resurgence in the last quarter of the 20th century, spreading from its historic Asiatic locale to the Americas, including the coastline of the United States.

## VIBRIO

Vibrios are curved, Gram-negative rods commonly found in saltwater. Cells may be linked end to end, forming S shapes and spirals. They are highly motile with a single polar flagellum, non–spore forming, oxidase positive, and can grow under aerobic or anaerobic conditions. The cell envelope structure is similar to that of other Gram-negative bacteria. *Vibrio cholerae* is the prototype cause of a water-loss diarrhea called **cholera.** Other species causing diarrhea, wound infections, and, rarely, systemic infection are listed in Table 22–2.

Rapidly motile curved rods are found in seawater

## *Vibrio cholerae*

### BACTERIOLOGY

#### GROWTH AND STRUCTURE

*V. cholerae* has a low tolerance for acid, but grows under alkaline (pH 8.0 to 9.5) conditions that inhibit many other Gram-negative bacteria. It is distinguished from other vibrios by its biochemical reactions, lipopolysaccharide (LPS) O antigenic structure, and production of cholera toxin (CT). There are over 150 O antigen serotypes, only two of

TABLE 22–1

**Features of *Vibrio, Campylobacter,* and *Helicobacter*[a]**

| Organism | Bacteriology: Growth | Bacteriology: Urease | Epidemiology | Pathogenesis | Disease |
|---|---|---|---|---|---|
| *Vibrio cholerae* | Facultative | – | Fecal–oral, water-borne, pandemics | Cholera toxin (Ace, Zot) | Watery diarrhea (cholera) |
| *Campylobacter jejuni* | Microaerophilic | – | Animals, unpasteurized dairy products | Unknown | Dysentery, watery diarrhea |
| *Helicobacter pylori* | Microaerophilic | + | Unknown | Vacuolating cytotoxin, urease | Chronic gastritis, ulcers, adenocarcinoma, lymphoma |

[a]All are curved Gram-negative rods with similar morphology.

*Abbreviations:* Ace, accessory cholera enterotoxin; Zot, zona occludens toxin (loosens tight junctions).

TABLE 22–2

**Features of Less Common *Vibrio* and *Campylobacter* Species**

| Organism | Features | Epidemiology | Disease |
|---|---|---|---|
| ***Vibrio*** | | | |
| *V. mimicus* | Closely related to *V. cholerae,* cholera-like enterotoxin | Ingestion of raw seafood | Watery diarrhea |
| *V. parahaemolyticus* | Produces bowel inflammation, enterotoxin unclear | Coastal seawater; ingesting raw seafood; outbreaks on cruise ships; common in Japan | Watery diarrhea, occasionally dysentery |
| *V. vulnificus* | Produces powerful siderophores which scavenge iron from host transferrin and lactoferrin | Coastal seawater, particularly when water temperatures rise; ingesting raw seafood or contamination of wound with seawater | Fulminant bacteremia following ingestion, cellulitis from wound contamination, high fatality in those with $Fe^{+}$ storage disease |
| *V. alginolyticus* | | Wounds contaminated by seawater | Cellulitis |
| ***Campylobacter*** | | | |
| *C. fetus* | Fails to grow on selective medium used for *C. jejuni* | Cause of abortion in cattle and sheep | Bacteremia, thrombophlebitis |
| *C. upsaliensis* | Fails to grow on selective medium used for *C. jejuni* | Associated with dogs and cats | Diarrhea similar to *C. jejuni* |
| *C. hyointestinalis* | | Enteritis in swine | Diarrhea in immunocompromised and homosexual men |
| *C. lari* | | Associated with birds | Diarrhea, bacteremia in immunocompromised |

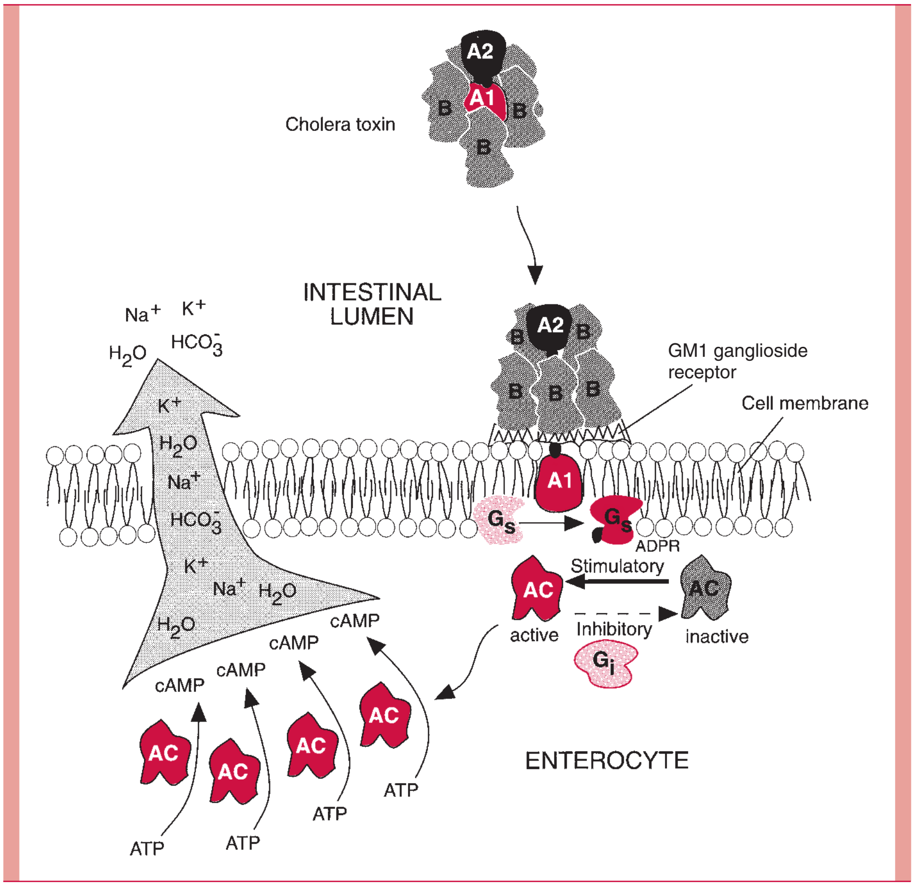


FIGURE 22–1
The action of cholera toxin. The complete toxin is shown binding to the GM1-ganglioside receptor on the cell membrane via the binding (B) subunits. The active portion (A1) of the A subunit catalyzes the ADP-ribosylation of the $G_S$ (stimulatory) regulatory protein, "locking" it in the active state. Because the $G_S$ protein acts to return adenylate cyclase from its inactive to active form, the net effect is persistent activation of adenylate cyclase. The increased adenylate cyclase activity results in accumulation of cyclic adenosine 3′,5′-monophosphate (cAMP) along the cell membrane. The cAMP causes the active secretion of sodium ($Na^+$), chloride ($Cl^-$), potassium ($K^+$), bicarbonate ($HCO_3^-$), and water out of the cell into the intestinal lumen.

which (O1 and O139) cause cholera. An O1 variant, *V. cholerae* biogroup El Tor is distinguished by biochemical reactions. O139 strains resemble O1 El Tor strains but possess a unique O antigen and have a polysaccharide capsule. *V. cholerae* possess long filamentous pili that form bundles on the bacterial surface and belong to a family of pili whose chemical structure is similar to those of the gonococcus, and a number of other bacterial pathogens. All strains capable of causing cholera produce a colonizing factor known as the toxin-coregulated pilus (TCP) because its expression is regulated together with CT.

Growth prefers alkaline over acid conditions

Cholera is limited to O1 and O139 serotypes

Serotype O139 is encapsulated

## CHOLERA TOXIN

The structure and mechanism of action of CT has been studied extensively (Fig 22–1). CT is an A-B type ADP-ribosylating toxin. Its molecule is an aggregate of multiple polypeptide chains organized into two toxic subunits (A1, A2) and five binding (B) units. The B units bind to a GM1-ganglioside receptor found on the surface of many types of cells. Once bound, the A1 subunit is released from the toxin molecule by reduction of the disulfide bond that binds it to the A2 subunit, and it enters the cell by translocation. In the cell, it exerts its effect on the membrane-associated adenylate cyclase system at the basolateral membrane surface. The target of the toxic A1 subunit is a guanine nucleotide (G) protein, Gs$\alpha$, that regulates activation of the adenylate cyclase system. CT catalyzes the ADP ribosylation of the G protein, rendering it unable to dissociate from the active adenylate cyclase complex. This causes persistent activation of intracellular adenylate cyclase, which in turn stimulates the conversion of adenosine triphosphate to cyclic adenosine 3′, 5′-monophosphate (cAMP). The net effect is excessive accumulation of cAMP at the cell membrane, which causes hypersecretion of chloride, potassium, bicarbonate, and associated water molecules out of the cell. Strains of *V. cholerae* other than the two epidemic serotypes may or may not produce CT.

B subunit receptor is a ganglioside on cell surface

A1 enters cytoplasm and ADP-ribosylates regulatory G protein

Adenylate cyclase becomes locked in active state

Hyperproduction of cAMP causes hypersecretion of water and electrolytes

# CHOLERA

CLINICAL CAPSULE

Cholera produces the most dramatic watery diarrhea known. Intestinal fluids pour out in voluminous bowel movements; this eventually leads to dehydration and electrolyte imbalance. These effects come from the action of cholera toxin secreted by *V. cholerae* in the bowel lumen. Despite the profound physiologic effects, there is no fever, inflammation, or direct injury to the bowel mucosa.

## EPIDEMIOLOGY

Transmission is through untreated water supply

Incubation period is 2 days

Epidemic cholera is spread primarily by contaminated water under conditions of poor sanitation, particularly where sewage treatment is absent or defective. Even though convalescent human carriage is brief, if the numerous vibrios purged from the intestines of cases are able to reach the primary water supply, the conditions for spread are established. The short incubation period (2 days) ensures that organisms ingested by others quickly enter the epidemic cycle. Even so, modern travel makes imported cases possible. One man developed diarrhea in Florida after eating ceviche (marinated uncooked fish) just before departure from an airport in Ecuador.

Cholera is endemic in India and Africa

Pandemics span decades

Gulf Coast cases result from undercooked shellfish

Latin American epidemic is widespread

Cholera is endemic in the Indian subcontinent and Africa. Over the past two centuries, its spread beyond this historic locale to other parts of Asia, Indonesia, and Europe has been described in eight great pandemics, each lasting 5 to 25 years. The current pandemic has brought cholera to the Western Hemisphere for the first time since 1911. Sporadic cases in the United States first appeared in the early 1970s and were traced to inadequately cooked crabs and shrimp caught off the Gulf Coast of Louisiana and Texas. In 1991, Latin America was hit with epidemic cholera with cases reported from 21 countries from Peru to northern Mexico. In Peru alone, over 500,000 cases and 4500 deaths occurred in 2 years. The disease is now endemic, claiming thousands of lives every year. Virulent *V. cholerae* now lurks in coastal waters throughout the hemisphere and in the drinking water of locales with poor sanitation.

El Tor biotype dominated 20th century

New O139 serotype is spreading

The dominant strain of the 20th century was the El Tor biotype, first isolated from Mecca pilgrims at the El Tor quarantine camp in 1905. This strain survives slightly longer in nature and is more likely to produce subclinical cases of cholera, both of which facilitated its spread. In 1992, the first cholera cases due to a serotype other than O1 were detected in India and Bangladesh. The new serotype (O139 Bengal) is fully virulent with the additional threat of enhanced ability to produce disease in persons whose immunity is due to exposure to the old serotype. This development is important for the global spread of cholera and for the vaccine strategies designed to prevent it.

Dormant form in plankton may facilitate interepidemic survival

The triggering of epidemics and the interepidemic survival of *V. cholerae* in the environment is incompletely understood but may be linked to **crustaceans** and the **plankton** population. *V. cholerae* in a dormant state can be demonstrated by immunofluorescence in plankton, and epidemics follow plankton blooms. Otherwise the organism is fragile, surviving only a few days in the environment unless maintained longer in marine and freshwater crustaceans.

## PATHOGENESIS

Large doses required to pass stomach acid barrier

Pili mediate epithelial adherence

CT-stimulated intestinal hypersecretion causes diarrhea

To produce disease, *V. cholerae* must reach the small intestine in sufficient numbers to multiply and colonize. In healthy people, ingestion of large numbers of bacteria is required to offset the acid barrier of the stomach. Colonization of the entire intestinal tract from the jejunum to the colon by *V. cholerae* requires organism adherence to the epithelial surface, most probably by surface pili. The outstanding feature of *V. cholerae* pathogenicity is the ability of virulent strains to secrete CT, which is responsible for the disease cholera. The water and electrolyte shift from the cell to the intestinal lumen is the fundamental cause of the watery diarrhea of cholera.

### Fluid Loss

The fluid loss that results from the adenylate cyclase stimulation of cells depends on the balance between the amount of bacterial growth, toxin production, fluid secretion, and fluid absorption in the entire gastrointestinal tract. The outpouring of fluid and electrolytes is greatest in the small intestine, where the secretory capacity is high and absorptive capacity low. The diarrheal fluid can amount to many liters per day, with approximately the same sodium content as plasma but two to five times the potassium and bicarbonate concentrations. The result is dehydration (isotonic fluid loss), hypokalemia (potassium loss), and metabolic acidosis (bicarbonate loss). The intestinal mucosa remains unaltered except for some hyperemia, because *V. cholerae* does not invade or otherwise injure the enterocyte. Mutants lacking CT may still cause mild diarrhea due to recently discovered accessory toxins which cause fluid secretion or increase intestinal permeability.

Small intestine loses liters of fluid

$K^+$ and bicarbonate losses cause hypokalemia and acidosis

Intestinal mucosa is structurally unaffected; no invasion

### Genetic Regulation of Virulence

The expression of the multiple virulence factors of *V. cholerae* is controlled in a complex but coordinated system involving environmental sensors and as many as 20 chromosomal genes divided between a pathogenicity island (PAI) containing CT and one containing TCP. The chief regulator is a transmembrane protein (ToxR ) that "senses" environmental changes in pH, osmolarity, and temperature which convert it to an active form. In the active state, ToxR can directly turn on CT genes as well as activate transcription of a second regulatory protein, ToxT. ToxT can then activate transcriptional of virulence genes in both PAIs, including TCP, CT, and accessory toxins.

Regulatory system turns on CT and TCP in response to environmental changes

## IMMUNITY

Nonspecific defenses such as gastric acidity, gut motility, and intestinal mucus are important in preventing colonization with *V. cholerae*. For example, in persons who lack gastric acidity (gastrectomy or achlorhydria from malnutrition), the attack rate of clinical cholera is higher. Natural infection provides long-lasting immunity. The immune state has been associated with IgG directed against the cell wall LPS and with the production of secretory IgA by lymphocytes in the subepithelial areas of the gastrointestinal tract. The precise protective mechanisms remain to be established.

Attack rate is higher with achlorhydria

Immunity is associated with sIgA

# CHOLERA: CLINICAL ASPECTS

## MANIFESTATIONS

Typical cholera has a rapid onset, beginning with abdominal fullness and discomfort, rushes of peristalsis, and loose stools. Vomiting may also occur. The stools quickly become watery, voluminous, almost odorless, and contain mucus flecks, giving it an appearance called **rice-water stools.** Neither white blood cells or blood are present in the stools, and the patient is afebrile. Clinical features of cholera result from the extensive fluid loss and electrolyte imbalance, which can lead to extreme dehydration, hypotension, and death within hours if untreated.

Extreme watery diarrhea causes large fluid loss

Dehydration and electrolyte imbalance are the major problems

## DIAGNOSIS

The initial suspicion of cholera depends on recognition of the typical clinical features in an appropriate epidemiologic setting. A bacteriologic diagnosis is accomplished by isolation of *V. cholerae* from the stool. The organism grows on common clinical laboratory media such as blood agar and MacConkey agar, but its isolation is enhanced by the use of a selective medium (thiosulfate-citrate-bile salt-sucrose agar). Once isolated, the organism is readily identified by biochemical reactions. Outside cholera endemic areas, the

Stool culture using selective media is required selective medium is not routinely used for stool cultures, so clinical laboratories must be alerted to the suspicion of cholera.

## TREATMENT

Oral or intravenous fluid and electrolyte replacement is crucial

Antimicrobic therapy can reduce duration and severity

The outcome of cholera is dependent on balancing the diarrheal fluid and ionic losses with adequate fluid and electrolyte replacement. This is accomplished by oral and/or intravenous administration of solutions of glucose with near physiologic concentrations of sodium and chloride and higher than physiologic concentrations of potassium and bicarbonate. Exact formulas are available as dried packets to which a given volume of water is added. Oral replacement, particularly if begun early, is sufficient for all but the most severe cases and has substantially reduced the mortality from cholera. Antimicrobial therapy plays a secondary role to fluid replacement. Tetracyclines shorten the duration of diarrhea and magnitude of fluid loss. Trimethoprim–sulfamethoxazole and erythromycin are alternatives for use in children and pregnant women.

## PREVENTION

Water sanitation and cooking shellfish prevent infection

Vaccines are disappointing

Epidemic cholera, a disease of poor sanitation, does not persist where treatment and disposal of human waste is adequate. Because good sanitary conditions do not exist in much of the world, secondary local measures such as boiling or chlorination of water during epidemics are required. The cases associated with crustaceans can be prevented by adequate cooking (10 minutes) and avoidance of recontamination from containers and surfaces. Vaccines prepared from whole cells, lipopolysaccharide, and CT B subunit have been disappointing, providing protection that is not long-lasting. Current interest includes live attenuated vaccine strains because of their potential to stimulate the local sIgA immune response.

# *Other Vibrios*

*V. parahaemolyticus* in undercooked or raw seafood causes diarrhea

*V. vulnificus* sepsis and wound infections linked to raw oysters and iron overload

Species of *Vibrio* other than *V. cholerae* may still produce disease but are uncommon and typically restricted to seacoast locales. Most, such as *V. parahaemolyticus,* produce a diarrheal illness following ingestion of raw or inadequately cooked seafood. They do not produce cholera toxin, but some have been shown to produce their own enterotoxins. Of these, *V. vulnificus* stands out because it can produce a rapidly progressive cellulitis in wounds sustained in seawater and a bacteremic infection following ingestion of raw seafood. The latter has a high mortality rate and has been common enough in Florida to threaten the local oyster trade. *V. vulnificus* has also been shown to be a spectacular scavenger of host iron stores and produces particularly fulminant disease in persons with iron-overload states (eg, thalassemia, hemochromatosis). Features of the less common vibrios are included in Table 22–2.

# *CAMPYLOBACTER*

Campylobacters are motile, curved, oxidase-positive, Gram-negative rods similar in morphology to vibrios. The cells have polar flagella and are often are attached at their ends giving pairs "S" shapes or a "seagull" appearance. More than a dozen *Campylobacter* species have been associated with human disease. Of these *C. jejuni,* and *C. coli* are by far the most common and similar enough to be considered as one. Some other *Campylobacter* species are potential causes of diarrhea, but only *C. jejuni* will be discussed here. The features of other species are summarized in Table 22–2.

## BACTERIOLOGY: *Campylobacter jejuni*

Before 1973, *C. jejuni* was not recognized as a cause of human disease. It was not until selective methods for its isolation were developed that it was recognized as one of the most common causes of infectious diarrhea. Like other campylobacters, *C. jejuni* grows well only on enriched media under microaerophilic conditions. That is, it requires oxygen at reduced tension (5–10%), presumably due to vulnerability of some of its enzyme systems to superoxides. Growth usually requires 2 to 4 days, sometimes as much as a week. *C. jejuni* has the structural components found in other Gram-negative bacteria (eg, outer membrane, LPS). In contrast to the vibrios, it does not break down carbohydrates, but uses amino acids and metabolic intermediates for energy.

Microaerophilic atmosphere is required for growth

## CAMPYLOBACTER ENTERITIS

CLINICAL CAPSULE

*C. jejuni* infection typically begins with lower abdominal pain, which evolves into diarrhea over a matter of hours. The diarrhea may be watery or dysenteric, with blood and pus in the stool. Most patients are febrile. The illness resolves spontaneously after a few days to 1 week.

### EPIDEMIOLOGY

It is humbling to consider how a pathogen as common as *C. jejuni* could have been missed for decades. Studies from many countries find *C. jejuni* in 4 to 30% of diarrheal stools, making it the leading cause of gastrointestinal infection in developed countries. Over 2 million cases occur each year in the United States, at a rate roughly double the second most common bacterial enteric pathogen, *Salmonella.* This high rate of disease is facilitated by the low infecting dose of *C. jejuni*—only a few hundred cells.

Causes diarrhea worldwide

Infecting dose is low

The primary reservoir is in animals and the bacteria are transmitted to humans by ingestion of contaminated food or by direct contact with pets. Campylobacters are commonly found in the normal gastrointestinal and genitourinary flora of warm-blooded animals, including sheep, cattle, chickens, wild birds, and many others. Domestic animals such as dogs may also carry the organisms and probably play a significant role in transmission to humans. The most common source of human infection is undercooked poultry, but outbreaks have been caused by contaminated rural water supplies and unpasteurized milk often consumed as a "natural" food. Sometimes a direct association can be made as with a sick household pet.

Reservoir is animals

Undercooked poultry and unpasteurized milk are major sources

### PATHOGENESIS

Infection is established by oral ingestion, followed by colonization of the intestinal mucosa. The bacteria have been shown to adhere to endothelial cells and then enter cells in endocytotic vacuoles. Once inside, they move in association with the cell's microtubule structure, rather than the actin microfilaments associated with many other invasive bacteria. The search for enterotoxins associated with *C. jejuni* has thus far been unrewarding. A cytolethal distending toxin arrests cell division while the cytoplasm continues to grow, but how this leads to diarrhea remains to be established. All in all, the virulence determinants of this microorganism remain uncertain.

Intracellular movement is associated with microtubules

Cytolethal distending toxin is a candidate

There is an association between *C. jejuni* infection and the **Guillain-Barré syndrome,** an acute demyelinating neuropathy that is frequently preceded by an infection. Although *C. jejuni* is not the only antecedent to this syndrome, it is the most common of identifiable causes. Up to 40% of patients have culture or serologic evidence of *Campylobacter* infection at the time the neurologic symptoms occur. The mechanism is believed to involve antibody elicited by ganglioside-like structures in the *C. jejuni* LPS core

Guillain-Barré syndrome may follow infection

Antiganglioside antibodies cross-react with neural tissue

oligosaccharide that cross-react with similar molecules in the host peripheral nerve myelin. These antiganglioside antibodies are found in the serum of patients with Guillain-Barré syndrome motor neuropathies. This molecular mimicry is similar to the mechanism of rheumatic fever stimulated by the group A streptococcus (see Chapter 17).

## IMMUNITY

Immune mechanisms are unclear

Acquired immunity following natural infection with *C. jejuni* has been demonstrated in volunteer studies, but the mechanisms involved are unknown. Antibodies are formed in the weeks following infection but decline rapidly thereafter. The high rate of *Campylobacter* infection in patients with acquired immunodeficiency syndrome suggests the importance of cellular immune mechanisms.

# CAMPYLOBACTEROSIS: CLINICAL ASPECTS

## MANIFESTATIONS AND DIAGNOSIS

Abdominal pain and dysentery are present

Selective medium is incubated in microaerophilic atmosphere

The illness typically begins 1 to 7 days after ingestion, with fever and lower abdominal pain that may be severe enough to mimic acute appendicitis. These are followed within hours by dysenteric stools that usually contain blood and pus. The illness is typically self-limiting after 3 to 5 days but may last 1 to 2 weeks. The diagnosis is confirmed by isolation of the organism from the stool. This requires a special medium made selective for *Campylobacter* by inclusion of antimicrobics that inhibit the normal facultative flora of the bowel. Plates must be incubated in a microaerophilic atmosphere that can now be conveniently generated in a sealed jar by hydration of commercial packs similar to those used for anaerobes.

## TREATMENT

Erythromycin may shorten course

Since less than half of patients clearly benefit from antimicrobial therapy, cases are usually not treated unless the disease is severe or prolonged (>1 week). *C. jejuni* is typically susceptible to macrolides and fluoroquinolones but resistant to $\beta$-lactams. Erythromycin is considered the treatment of choice but must be given early for maximal effect. Fluoroquinolones are also effective, but resistance is becoming more common.

# *HELICOBACTER*

Everything we once knew about ulcers was wrong

In 1983, a pair of Australian microbiologists suggested that gastritis and peptic ulcers were infectious diseases, contradicting long-held beliefs concerning their epidemiology, pathogenesis, and treatment. In the same year, the 10th edition of *Harrison's Principles of Internal Medicine* described peptic ulcers as due to an unfavorable balance between gastric acid–pepsin secretion and gastric or duodenal mucosal resistance. Underlying causes cited included genetic and lifestyle (smoking) as well as psychological factors (anxiety, stress). Treatment with bismuth salts, antacids, and inhibitors of acid secretion gave relief but not cure. Relapsing patients (50 to 80%) were subjected to surgical treatments (vagotomy, partial gastrectomy), which had their own set of complications (reflux, afferent loop syndrome, dumping syndrome). All of this was logical and supported by clinical observations and research studies. It was simply incorrect. The bacteria now called *Helicobacter* had been observed but dismissed because they were so common and its urease was once considered a secretory product of the stomach itself. The paper by Warren and Marshall (see Additional Reading) stimulated the reversal, which has led to cures using antimicrobics and new ideas linking *Helicobacter* infection to cancer. This experience has also left us with a sense that we can never be smug about what we "know" in medicine.

## BACTERIOLOGY: *Helicobacter pylori*

*H. pylori* has morphologic and growth similarities to the campylobacters, with which they were originally classified. The cells are slender, curved rods with polar flagella. The cell wall structure is typical of other Gram-negative bacteria, although *Helicobacter* LPS may be less toxic than its enteric counterparts. Growth requires a microaerophilic atmosphere and is slow (3 to 5 days).

Features are similar to *Campylobacter* including microaerophilic requirement

A number of unique bacteriologic features have been found in *H. pylori.* The most distinctive is a **urease** whose action allows the organism to persist in low pH environments by the generation of ammonia. The urease is produced in amounts so great (6% of bacterial protein) that its action can be demonstrated within minutes of placing *H. pylori* in the presence of urea. Another secreted protein called the **vacuolating cytotoxin** (VacA) causes apoptosis in eukaryotic cells it enters generating multiple large cytoplasmic vacuoles. The vacuoles are felt to be generated by the toxin's formation of channels in lysosomal and endosomal membranes.

Urease raises pH rapidly

VacA injures lysosomal and endosomal membranes

Most *H. pylori* strains also contain a 30+ gene PAI, so called because the guanine + cytosine content of the PAI differs from the rest of the genome. This suggests the PAI is a genetic cassette acquired from some unknown organism in the distant past. Most of the PAI genes code for elements of a **contact secretion system,** which in other bacteria transfers DNA or proteins across the outer membrane to the extracellular space or into other cells. The cells receiving the products of these secretion systems include bacterial, plant, and epithelial cells. In *H. pylori,* the secretion system injects VacA and a protein *Cag*, also coded in the PAI, into epithelial cells. Once in the cell, *Cag* induces changes in multiple cellular proteins and has a strong association with virulence (see Pathogenesis).

PAI contains genes for *Cag* and contact secretions system

*Cag* induces changes inside host cell

## *HELICOBACTER* GASTRITIS

CLINICAL CAPSULE

*Helicobacter* infections are limited to the mucosa of the stomach, and most are asymptomatic even after many years. Burning pain in the upper abdomen, accompanied by nausea and sometimes vomiting, is a symptom of gastritis. Ulcers may cause additional symptoms, depending on their anatomic location. It is common for gastric and duodenal ulcers to be unrecognized by the patient until they cause frank bleeding or rupture.

### EPIDEMIOLOGY

Infection with *H. pylori* causes what is perhaps the most prevalent disease in the world. The organism is found in the stomachs of 30 to 50% of adults in developed countries and it is almost universal in developing countries. The exact mode of transmission is not known, but is presumed to be person to person by the fecal–oral route or by contact with gastric secretions in some other way. Colonization increases progressively with age, and children are believed to be the major amplifiers of *H. pylori* in human populations. A declining prevalence in developed countries may be due to decreased transmission because of less crowding and frequent exposure to antimicrobics.

Infection is transmitted by human fecal or gastric secretions

Gastric colonization is prevalent worldwide

Once established, the same strain persists at least for decades, probably for life. Molecular epidemiologic analysis indicates the strains themselves have strong linkages to ethnic origins that can be traced back to the earliest known patterns of human migration. *H. pylori* has been called an "accidental tourist," which was established in the stomachs of

Colonization persists indefinitely

Ethnic links are strong

humans before migration began and remained bound to the original population as it dispersed from continent to continent over thousands of years.

H. pylori is the sole nondrug cause of gastritis and ulcers

Adenocarcinoma and lymphoma are preceded by infection

*H. pylori* is the most common cause of gastritis, gastric ulcer, and duodenal ulcer. In addition *Helicobacter* gastritis caused by $Cag^{+}$ strains is acknowledged to be the antecedent cause of gastric adenocarcinoma, one of the most common causes of cancer death in the world. It is also linked to a gastric mucosa-associated lymphoid tissue (MALT) lymphoma, which is less common but shows the striking property of regressing with antimicrobial therapy. *H. pylori* recently gained the dubious distinction of being the first bacterium declared a class I carcinogen by the World Health Organization.

Other *Helicobacter* species occur in animals

*H. pylori* is exclusive to humans, but other species have been found in the stomachs of a wide range of animals, where they are also associated with gastritis. It is difficult to imagine the old "stress ulcer" theories surviving the discovery of a cheetah with *Helicobacter* gastritis. Speculation that domestic animals may serve as a reservoir for human infection has not been confirmed.

## PATHOGENESIS

Urease ammonia production neutralizes acid

Motility facilitates surface microenvironment

In order to persist in the hostile environs of the stomach, *H. pylori* employs multiple mechanisms to adhere to the gastric mucosa and survive the acid milieu of the stomach. Motility provided by the flagella allows the organisms to swim to the less acid pH locale beneath the gastric mucus, where the urease further creates a more neutral microenvironment by ammonia production. At the mucosa, adherence is mediated by surface proteins, one of which binds to Lewis blood group antigens, often present on the surface of gastric epithelial cells.

VacA and *Cag* stimulate inflammation

*H. pylori* colonization is virtually always accompanied by a cellular infiltrate ranging from minimal mononuclear infiltration of the lamina propria to extensive inflammation with neutrophils, lymphocytes, and microabscess formation. This inflammation may be due to toxic effects of the urease or the VacA. The *Cag* protein may contribute by stimulation of cytokines (interleukin-8), and a neutrophil-activating protein (NAP) has been shown to recruit neutrophils to the gastric mucosa. Added together urease, *Cag*, and NAP provide ample explanation for the gastritis that is universal in *H. pylori* infection.

VacA directly induces cellular changes and death

*Cag* has strong association but uncertain role

A prolonged and aggressive inflammatory response could lead to epithelial cell death and ulcers, but other virulence factors play a more direct role. The chief of these is VacA, which is responsible for much of the epithelial cell erosion seen in human infection. The vacuolar degeneration it induces is readily visible histologically in gastric biopsies (Fig 22–2). The importance of *Cag* is clear from its epidemiologic association with ulcers, but its exact role is unclear. When *Cag* is transported into epithelial cells by the PAI secretion system, it induces an active reorganization of the cellular actin cytoskeleton and activation of multiple host cell proteins. How these changes are integrated to contribute to ulcer formation remains to be demonstrated.

Carcinogenic mechanisms are unknown

That decades of inflammation and assault by the virulence factors described above could eventually lead to cancer seems logical, but the specific mechanisms of carcinogenesis are unknown. *Cag* is a leading candidate. A curious paradox is that while $Cag^{+}$ strains are associated with ulcers and adenocarcinoma of the lower stomach, they are associated with a decreased incidence of adenocarcinoma of the upper stomach (cardia) and esophagus. Dissection of the many actions *Cag* has within cells should shed light on these issues.

# *HELICOBACTER* DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

Epigastric pain and nausea are signs of gastritis

Primary infection with *H. pylori* is either silent or causes an illness with nausea and upper abdominal pain lasting up to 2 weeks. Years later, the findings of gastritis and peptic ulcer disease include nausea, anorexia, vomiting, epigastric pain, and even less specific symptoms

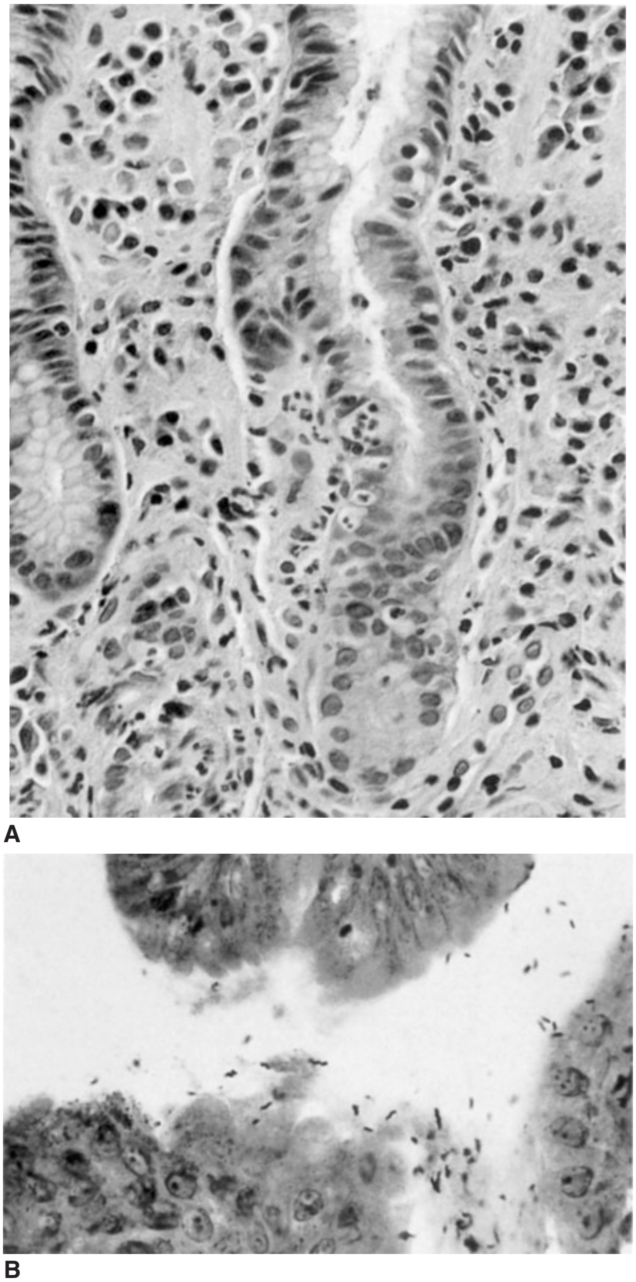


FIGURE 22–2
*Helicobacter* gastritis. **A.** Gastric mucosa shows infiltration of neutrophils and destruction of epithelial cells. **B.** High magnification shows curved bacilli. (*Reproduced with permission from Connor DH, Chandler FW, Schwartz DQA, Manz HJ, Lack EE (eds).* Pathology of Infectious Diseases, *vol. 1. Stamford, CT: Appleton & Lange; 1997.*)

such as belching. Many patients are asymptomatic for decades, even up to perforation of an ulcer. Perforation can lead to extensive bleeding and peritonitis due to the leakage of gastric contents into the peritoneal cavity.

## DIAGNOSIS

Urease detection is diagnostic

Serologic tests demonstrate chronic infection

The most sensitive means of diagnosis is endoscopic examination, with biopsy and culture of the gastric mucosa. The *H. pylori* urease is so potent its activity can be directly demonstrated in biopsies in less than an hour. Noninvasive methods include serology and a urea breath test. For the breath test, the patient ingests $^{13}C$- or $^{14}C$-labeled urea, from which the urease in the stomach produces products that appear as labeled $CO_2$ in the breath. A number of methods for detection of antibody directed against *H. pylori* are now available. Because IgG or IgA remain elevated as long as the infection persists, these tests are valuable both for screening and for evaluation of therapy. The advantage of direct detection of the organism is that culture is the most sensitive indicator of cure following therapy.

## TREATMENT AND PREVENTION

Antimicrobics and bismuth salts achieve lasting cures

Regimen may be difficult to tolerate

*H. pylori* is susceptible to a wide variety of antimicrobial agents. Bismuth salts (eg, Pepto-Bismol), which in the past were believed to act by coating the stomach, also have antimicrobial activity. Cure rates approaching 95% have been achieved with various combinations of bismuth salts and two antibiotics. Metronidazole, tetracycline, clarithromycin, and amoxicillin have been effective. Relapse rates are low, particularly when acid secretion is also controlled with the use of a proton pump inhibitor. These combination regimens must be continued for at least 2 weeks and may be difficult for some patients to tolerate. Prevention of *H. pylori* disease awaits further understanding of transmission and immune mechanisms. Prophylactic treatment of asymptomatic persons colonized with *H. pylori* is not yet recommended.

## ADDITIONAL READING

Blake PA, Allegra DT, Snyder JD, et al: Cholera—A possible endemic focus in the United States. *N Engl J Med* 1980;302:305–309. This study was the first to detail the epidemiologic features of cholera cases along the Gulf Coast of Louisiana.

Covacci A, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R. *Helicobacter pylori* virulence and genetic geography. *Science* 1999;284:1328–1333. This paper provides an elegant argument for *H. pylori* as the most prevalent and ancient of infectious diseases.

Dunn BE, Cohen H, Blaser MJ. *Helicobacter pylori. J Clin Microbiol* 1997;10:720–741. This very readable and well-referenced review of all aspects of *Helicobacter* disease includes discussion of pathogenesis and treatment and some comments about animal helicobacters.

Farque SM, Albert, Mekalanos JJ: Epidemiology, genetics, and ecology of toxigenic *Vibrio cholerae. Microbiol Mol Biol Rev* 1998;62:1301–1314. This review mixes a discussion of the historic pandemics with a clear explanation of the complex regulation of virulence factors of *V. cholerae.* It concludes with some provocative ideas about how the cholera vibrio emerges from its mysterious environmental reservoir.

Nachamkin I, Alos BM, Ho T. *Campylobacter* species and Guillain-Barré syndrome. *Clin Microbiol Rev* 1998;11:555–567. This review gives a detailed examination of the molecular mimicry model for the connection between Guillain-Barré syndrome and *C. jejuni.*

Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;1:1273–1275. This paper led to the recognition of gastritis and ulcers as infectious diseases.

CHAPTER 23

# *Pseudomonas* and Other Opportunistic Gram-negative Bacilli

KENNETH J. RYAN

A number of opportunistic Gram-negative rods of several genera not considered in other chapters are included here. With the exception of *Pseudomonas aeruginosa,* they rarely cause disease, and all are frequently encountered as contaminants and superficial colonizers. The significance of their isolation from clinical material thus depends on the circumstance and site of culture and on the clinical situation of the patient.

## PSEUDOMONAS

There are a large number of *Pseudomonas* species, the most important of which is *P. aeruginosa*. The total number of infections produced by the other species is far lower than that produced by *P. aeruginosa* alone. *Pseudomonas* species are most frequently seen as colonizers and contaminants but are able to cause opportunistic infections. The assignment of species names has little clinical importance beyond differentiation from *P. aeruginosa*. Reports vary regarding the frequency of their isolation from cases of bacteremia, arthritis, abscesses, wounds, conjunctivitis, and urinary tract infections. In general, unless isolated in pure culture from a high-quality (direct) specimen, it is difficult to attach pathogenic significance to any of the miscellaneous *Pseudomonas* species.

*P. aeruginosa* most important

Other *Pseudomonas* species cause opportunistic infection

## *Pseudomonas aeruginosa*

### BACTERIOLOGY

*Pseudomonas aeruginosa* is an aerobic, motile, Gram-negative rod that is slimmer and more pale staining than members of the Enterobacteriaceae. Its most striking bacteriologic feature is the production of colorful water-soluble pigments. *P. aeruginosa* also

Pigment-producing rod is resistant to many antimicrobics demonstrates the most consistent resistance to antimicrobics of all the medically important bacteria.

## GROWTH AND METABOLISM

Grows aerobically with minimal requirements

Colonies are oxidase positive

*P. aeruginosa* is sufficiently versatile in its growth and energy requirements to use simple molecules such as ammonia and carbon dioxide as sole nitrogen and carbon sources. Thus, it does not require enriched media for growth, and it can survive and multiply over a wide temperature range (20 to 42°C) in almost any environment, including one with a high salt content. The organism uses oxidative energy-producing mechanisms and has a high level of cytochrome oxidase (oxidase positive). Although an aerobic atmosphere is necessary for optimal growth and metabolism, most strains multiply slowly in an anaerobic environment if nitrate is present as an electron acceptor.

Blue pyocyanin produced only by *P. aeruginosa*

Yellow fluorescin and pyocyanin combine for green color

Growth on all common isolation media is luxurious, and colonies have a delicate, fringed edge. Confluent growth often has a characteristic metallic sheen and emits an intense "fruity" odor. Hemolysis is usually produced on blood agar. The positive oxidase reaction of *P. aeruginosa* differentiates it from the Enterobacteriaceae, and its production of blue, yellow, or rust-colored pigments differentiates it from most other Gram-negative bacteria. The blue pigment, **pyocyanin,** is produced only by *P. aeruginosa*. **Fluorescin,** a yellow pigment that fluoresces under ultraviolet light, is produced by *P. aeruginosa* and other free-living less pathogenic *Pseudomonas* species. Pyocyanin and fluorescin combined produce a bright green color that diffuses throughout the medium.

## STRUCTURE

Outer membrane protein porins are relatively impermeable

Lipopolysaccharide (LPS) is present in the outer membrane, as are porin proteins, which differ from those of the Enterobacteriaceae in offering much less permeability to a wide range of molecules, including antibiotics. Pili composed of repeating monomers of the pilin structural subunit extend from the cell surface. A single polar flagellum rapidly propels the organism.

Secreted alginate forms a slime layer

Overproduction is due to regulatory mutations

A mucoid exopolysaccharide slime layer is present outside the cell wall in some strains. This layer is created by secretion of **alginate,** a copolymer of mannuronic and glucuronic acids. It is created by the action of several enzymes that effectively channel carbohydrate intermediates into the alginate polymer. All *P. aeruginosa* produce moderate amounts of alginate, but those with mutations in regulatory genes overproduce the polymer. These mutants appear as striking mucoid colonies in cultures from the respiratory tract of patients with cystic fibrosis.

## EXTRACELLULAR PRODUCTS

Multiple extracellular enzymes are produced

Exotoxin A action is same as diphtheria toxin

Most strains of *P. aeruginosa* produce multiple extracellular products, including **exotoxin A** and other enzymes with hemolytic, lecithinase, collagenase, or elastase activity. Exotoxin A enters cells via receptor-mediated endocytosis and is internalized into a low pH vesicle from which it translocates and reaches its target molecule, elongation factor 2 (EF-2). It catalyzes the inactivation of EF-2 by ADP-ribosylation, leading to shutdown of protein synthesis and cell death. Although this action is the same as diphtheria toxin, the two toxins are otherwise unrelated. Expression of exotoxin A is influenced by oxygen, temperature, and iron regulated genes.

Vimentin is ADP-ribosylated

**Exoenzyme S** ADP-ribosylates several intracellular proteins, including the cytoskeleton filament vimentin, and may also function as a surface-bound adhesin. The **elastase** acts on a variety of biologically important substrates, including elastin, human IgA and IgG, complement components, and some collagens. *P. aeruginosa* elastase shows homology with other proteases, including those produced by *Legionella pneumophila* and *Vibrio cholerae.*

## *P. aeruginosa* DISEASE

**Clinical Capsule**

*P. aeruginosa* produces infection at a wide range of pulmonary, urinary, and soft tissue sites, much like the opportunistic Enterobacteriaceae. The clinical manifestations of these infections reflect the organ system involved and are not unique for *Pseudomonas.* However, once established, infections are particularly virulent and difficult to treat. Affected patients almost always have some form of debilitation or compromise of immune defenses.

### EPIDEMIOLOGY

Primary habitat is environmental

Colonizes humans

The primary habitat of *P. aeruginosa* is the environment. It is found in water, soil, and various types of vegetation throughout the world. *P. aeruginosa* has been isolated from the throat and stool of 2 to 10% of healthy persons. Colonization rates may be higher in hospitalized patients. Infection with *P. aeruginosa,* rare in previously healthy persons, is one of the most important causes of invasive infection in hospitalized patients with serious underlying disease, such as leukemia, cystic fibrosis (CF), and extensive burns.

Multiplies in humidifiers, solutions, and medications

Risk for immunocompromised persons is high

The ability of *P. aeruginosa* to survive and proliferate in water with minimal nutrients can lead to heavy contamination of any nonsterile fluid, such as that in the humidifiers of respirators. Inhalation of aerosols from such sources can bypass the normal respiratory defense mechanisms and initiate pulmonary infection. Infections have resulted from the growth of *Pseudomonas* in medications, contact lens solutions, and even in some disinfectants. Sinks and faucet aerators may be heavily contaminated and serve as the environmental source for contamination of other items. The presence of *P. aeruginosa* in drinking water or food is not a cause for alarm. The risk lies in the proximity between items susceptible to contamination and patients uniquely predisposed to infection.

Respiratory colonization of CF patients becomes chronic

*P. aeruginosa* is now the most common bacterial pathogen to complicate the management of patients with CF, an inherited defect in chloride ion transport that leads to a buildup of thick mucus in ducts and the tracheobronchial tree. In a high proportion of cases, the respiratory tract becomes colonized with *P. aeruginosa,* which, once established, becomes almost impossible to eradicate. This infection is a leading cause of morbidity and eventual death of these patients.

### PATHOGENESIS

Needs break in first-line defenses

Pili, flagella, and slime mediate adherence

Although *P. aeruginosa* is an opportunistic pathogen, it is one of particular virulence. The organism usually requires a significant break in first-line defenses (such as a wound) or a route past them (such as a contaminated solution or intratracheal tube) to initiate infection. Attachment to epithelial cells is the first step in infection and is likely mediated by pili, flagella, and the extracellular polysaccharide slime. The receptors include sialic acid and N-acetylglucosamine borne by cell surface glycolipids. There is evidence that attachment is favored by loss of surface fibronectin, which explains in part the propensity for debilitated persons.

Extracellular enzymes are injected by contact secretion system

Exotoxin A is cytotoxic and immunogenic

Once established, the virulence of *P. aeruginosa* involves multiple factors, particularly exotoxin A, exotoxin S, and elastase, which are directly injected into host cells by a specialized contact secretion system. The importance of exotoxin A is supported by studies in human and animals, which correlate its presence with a fatal outcome and antibody against it with survival. No diphtheria-like systemic effect of exotoxin has been demonstrated, but its cytotoxic action correlates with the primarily invasive and locally destructive lesions seen in *P. aeruginosa* infections.

Elastin is attacked in lung and blood vessels

Exoenzyme S is associated with dissemination from burn wounds and with actions destructive to cells, including its action on the cytoskeleton. The many biologically important substrates of elastase argue for its importance, particularly its namesake, elastin. Elastin is found at some sites *P. aeruginosa* preferentially attacks, such as the lung and blood vessels. Hemorrhagic destruction, including the walls of blood vessels (Fig 23–1), is the histologic hallmark of *Pseudomonas* infection.

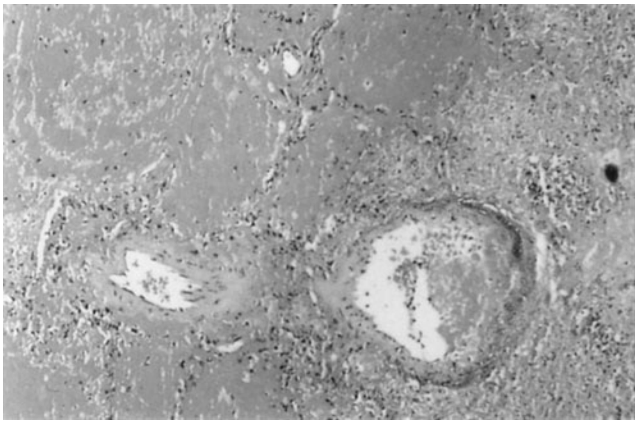

FIGURE 23–1
*Pseudomonas aeruginosa* pneumonia. This blood vessel in the lung of a fatal case is infected with *P. aeruginosa* and is undergoing destruction. A thrombus is forming in the lumen as well. (*Reproduced with permission from Connor DH, Chandler FW, Schwartz DQA, Manz HJ, Lack EE (eds).* Pathology of Infectious Diseases, *vol. 1. Stamford, CT: Appleton & Lange; 1997.*)

## *P. aeruginosa* and Cystic Fibrosis (CF)

Mutated strains overproduce alginate polymer

Glycocalyx biofilm protects bacteria

*P. aeruginosa* is the most persistent of the infectious agents that complicate the course of CF. Initial colonization may be aided by the fact that cells from CF patients are less highly sialylated than normal epithelial cells, providing increased receptors for *P. aeruginosa* attachment. Defects in the epithelia of CF patients may also retard their clearing by desquamation. Once the bronchi are colonized, the organisms remain, forming a biofilm containing microcolonies of bacteria, which together are called a **glycocalyx.** The most striking feature of this association is the unique presence of strains with multiple mutations in regulatory genes that cause overproduction of the alginate polymer. These genes are activated by the high osmolarity of the thick CF secretions. The selective advantages of this biofilm include adhesion; inaccessibility of the immune system (complement, antibody, phagocytes); and interference with the access and action of antimicrobial agents.

## Virulence Regulation

Multiple virulence factors are regulated by cell-to-cell signaling

The multiple virulence factors of *P. aeruginosa* are controlled by several regulatory pathways, some of which respond to environmental stimuli. In addition, some of the extracellular products, including exotoxin A and elastase, are regulated in an interactive way by cell-to-cell signaling. These signaling systems are able to monitor bacterial population density in a way that only initiates transcription of a virulence factor when certain population thresholds are reached. This could be valuable either as an economy measure or as a mechanism to withhold the onslaught of injury-producing molecules until the host has little time to respond.

## IMMUNITY

Humoral and cellular immune responses both important

Human immunity to *Pseudomonas* infection is not well understood. Inferences from animal studies and clinical observations suggest that both humoral and cell mediated immunity are important. The strong propensity of *P. aeruginosa* to infect those with defective cell-mediated immunity indicates that these responses are particularly important.

# *P. aeruginosa* DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

Infects burns and environmentally contaminated wounds

*P. aeruginosa* can produce any of the opportunistic extraintestinal infections caused by members of the Enterobacteriaceae. Burn, wound, urinary tract, skin, eye, ear, and respiratory infections all occur and may progress to bacteremia. *P. aeruginosa* is also one of the most common causes of infection in environmentally contaminated wounds (eg, osteomyelitis after compound fractures or nail puncture wounds of the foot).

*P. aeruginosa* pneumonia is a rapid, destructive, infection particularly in patients with granulocytopenia. It is associated with alveolar necrosis, vascular invasion, infarcts, and bacteremia. Pulmonary infection in CF patients is quite different; it is a chronic infection that alternates between a state of colonization and more overt bronchitis or pneumonia. Although the more aggressive features of *Pseudomonas* infection in the immunocompromised are not common, the infection is still serious enough to be a leading cause of death in CF patients.

Pneumonia is aggressive in the immunocompromised and chronic in CF

*P. aeruginosa* is also a common cause of otitis externa, including "swimmer's ear" and a rare but life-threatening "malignant" otitis externa seen in patients with diabetes. Folliculitis of the skin may follow soaking in inadequately decontaminated hot tubs that can become heavily contaminated with the organism. The organism can cause conjunctivitis, keratitis, or endophthalmitis when introduced into the eye by trauma or contaminated medication or contact lens solution. Keratitis can progress rapidly and destroy the cornea within 24 to 48 hours. In some cases of *P. aeruginosa* bacteremia, cutaneous papules develop that progress to black, necrotic ulcers. It is called **ecthyma gangrenosum** and is the result of direct invasion and destruction of blood vessel walls by the organism.

Common cause of otitis externa

Contamination of contact lenses leads to keratitis

Bacteremia may cause ecthyma gangrenosum

## DIAGNOSIS

*P. aeruginosa* is readily grown in culture. The combination of characteristic oxidase positive colonies, pyocyanin production and the ability to grow at 42°C is sufficient to distinguish *P. aeruginosa* from other *Pseudomonas* species. No other diagnostic modalities are in routine use.

Pigments typically produced in culture

## TREATMENT

Of the pathogenic bacteria, *P. aeruginosa* is the organism most consistently resistant to many antimicrobics. This is primarily due to the porins that restrict their entry to the periplasmic space. *P. aeruginosa* strains are regularly resistant to penicillin, ampicillin, cephalothin, tetracycline, chloramphenicol, sulfonamides, and the earlier aminoglycosides (streptomycin, kanamycin). Much effort has been directed toward the development of antimicrobics with anti-*Pseudomonas* activity. The newer aminoglycosides—gentamicin, tobramycin, and amikacin—are all active against most strains despite the presence of mutational and plasmid-mediated resistance. Carbenicillin and ticarcillin are active and can be given in high doses, but plasmid-mediated resistance and permeability mutations occur more frequently than with the aminoglycosides. The most prized feature of some of the third-generation cephalosporins (ceftazidime, cefepime, cefoperazone), carbapenems (imipenem, meropenem), and monobactams (aztreonam) is their activity against *Pseudomonas*. In general, urinary infections may be treated with a single drug, but more serious systemic *P. aeruginosa* infections are usually treated with a combination of an anti-*Pseudomonas* $\beta$-lactam antimicrobic and an aminoglycoside, particularly in neutropenic patients. Ciprofloxacin is also used in treatment of such cases. In all instances, susceptibility must be confirmed by in vitro tests.

Multiresistance is by restricting permeability

Mutational and plasmid mediated resistance occurs to penicillins and aminoglycosides

Third-generation cephalosporins are often active

The treatment of *P. aeruginosa* in CF presents special problems because most of the effective antimicrobics are only given intravenously. There is a reluctance to hospitalize in many patients, and oral agents are used instead. There is less experience with their efficacy under these conditions, and the chronic nature of CF is a set-up for development of resistance during therapy. This has already been seen with ciprofloxacin and aztreonam. Aerosolized tobramycin has also been used in some CF patients, with some evidence of clinical improvement.

Effective oral agents are scarce

## PREVENTION

Vaccines are experimental

Vaccines incorporating somatic antigens from multiple *P. aeruginosa* serotypes have been developed and proved immunogenic in humans. The primary candidates for such preparations are patients with burn injuries, CF, or immunosuppression. Although some protection has been demonstrated, these preparations are still experimental.

## BURKHOLDERIA

Melioidosis is a tropical pneumonia that relapses

*B. cepacia* is a nosocomial, CF pathogen

*Burkholderia pseudomallei* is a saprophyte in soil, ponds, rice paddies and vegetables located in Southeast Asia, the Philippines, Indonesia, and other tropical areas. Infection is acquired by direct inoculation or by inhalation of aerosols or dust containing the bacteria. The disease, **melioidosis,** is usually an acute pneumonia; however, it is sufficiently variable that subacute, chronic, and even relapsing infections may follow systemic spread. Some soldiers relapsed years after their return from Vietnam. The clinical and radiologic features may resemble tuberculosis. In fulminant cases, rapid respiratory failure may ensue and metastatic abscesses develop in the skin or other sites. Tetracycline, chloramphenicol, sulfonamides, and trimethoprim–sulfamethoxazole have been effective in therapy. *B. cepacia* is an opportunistic organism that has been found to contaminate reagents, disinfectants, and medical devices in much the same manner as *P. aeruginosa.* It has also complicated the course of CF but does not produce the mucoid colony type seen with *P. aeruginosa*.

## ACINETOBACTER

Respiratory and urinary infection come from soil and water

The genus *Acinetobacter* comprises Gram-negative coccobacilli that occasionally appear sufficiently round on Gram smears to be confused with *Neisseria*. On primary isolation, they closely resemble the Enterobacteriaceae in growth pattern and colonial morphology but are distinguished by their failure to ferment carbohydrates or reduce nitrates. As with most of the organisms discussed in this chapter, the isolation of *Acinetobacter* from clinical material does not define infection, because they appear most frequently as skin and respiratory colonizers. They are most frequently found as contaminants of almost anything wet, including soaps and some disinfectant solutions. Pneumonia is the most common infection, followed by urinary tract and soft tissue infections. Nosocomial respiratory infections have been traced to contaminated inhalation therapy equipment, and bacteremia to infected intravenous catheters. Treatment is complicated by frequent resistance to penicillins, cephalosporins, and occasionally aminoglycosides.

## MORAXELLA

Bronchitis and otitis come from respiratory flora

*Moraxella* is another genus of coccobacillary, Gram-negative rods that are usually paired end to end. Some species require enriched media, such as blood or chocolate agar. Their morphology, fastidious growth, and positive oxidase reaction can result in confusion with

*Neisseria*. This is particularly true for *M. catarrhalis,* which for many years was classified with *Neisseria*. More recently it was called *Branhamella catarrhalis*, and it is an occasional cause of otitis media and lower respiratory tract infection. Both infections relate to the presence of *M. catarrhalis* in the normal oropharyngeal flora. With the exception of *M. catarrhalis*, which frequently produces β-lactamase, *Moraxella* species are generally susceptible to penicillin.

## AEROMONAS AND PLESIOMONAS

The genera *Aeromonas* and *Plesiomonas* have bacteriologic features similar to those of the Enterobacteriaceae, *Vibrio,* and *Pseudomonas*. They are aerobic and facultatively anaerobic, attack carbohydrates fermentatively, and demonstrate various other biochemical reactions. *Aeromonas* colonies are typically β-hemolytic. The major taxonomic resemblance to *Pseudomonas* is that both *Aeromonas* and *Plesiomonas* are oxidase positive with polar flagella. Their habitat is basically environmental (water and soil), but they can occasionally be found in the human intestinal tract.

Resemble other enteric bacteria

*Aeromonas* is an uncommon but highly virulent cause of wound infections acquired in fresh or saltwater. The onset can be as rapid as 8 hours after the injury and the cellulitis progresses rapidly to fasciitis, myonecrosis, and bacteremia in less than a day. *Aeromonas* is also the leading cause of infections associated with the use of leeches, due to its regular presence in the leech foregut. In addition to opportunistic infection, some evidence suggests an occasional role for *Aeromonas* in gastroenteritis through production of toxins with enterotoxic and cytotoxic properties. *Plesiomonas* is also associated with an enterotoxic diarrhea. These associations are not yet strong enough to justify attempts to routinely isolate *Aeromonas* and *Plesiomonas* from diarrheal stools. Resistance to penicillins and cephalosporins is common. Most strains show susceptibility to tetracycline, with variable susceptibility to aminoglycosides, including gentamicin.

Rapid cellulitis follows injury in water

Diarrheas relate to enterotoxin production

## OTHER GRAM-NEGATIVE RODS

There are many other Gram-negative rods that rarely cause disease in humans. Some are members of the normal flora and others come from the environment. Because many of these do not ferment carbohydrates or react in many of the tests routinely used to characterize bacteria, their identification is frequently delayed as additional tests are tried or the organism is sent to a reference laboratory. The clinical significance of all these organisms is essentially the same; the clinician usually receives a report of a "nonfermenter" or another descriptive term and a susceptibility test result. The significance of the isolate is then determined on clinical grounds. The major characteristics of some of these organisms are shown in Table 23–1. The types of infection listed represent the most common among scattered case reports, and should not be interpreted as typical for each organism.

Rare species are interpreted on the basis of their clinical setting

Some Gram-negative bacilli fail to conform to any of the species currently recognized. If clinically important, such strains are sent to reference centers, such as the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. Eventually, some are given designations such as "CDC group IIF," which may appear in clinical reports. Much later, a new genus and/or species name may be issued if agreement among taxonomists is sufficient.

Some bacteria remain unnamed for years

TABLE 23–1

## *Pseudomonas* and other Opportunistic Gram-negative Rods

| | Bacteriologic Features | | | | | | |
|---|---|---|---|---|---|---|---|
| Species | MacConkey Growth | $CO_2$ Required | Pigments | Adherence | Virulence Factors | Epidemiology | Disease |
| ***Pseudomonas*** | | | | | | | |
| *P. aeruginosa* | + | – | Pyocyanin, fluorescin | Pili, flagella, alginate slime | Exotoxin A, exoenxyme S, elastase, alginate slime | Environmental, normal flora, mucosal breaks, nosocomial | Wounds, pneumonia, burns, otitis externa, cystic fibrosis |
| *P. fluorescens* | + | – | Fluorescin | | | Environmental | Opportunistic |
| Other species | + | – | Fluorescin | | | Environmental | Opportunistic |
| ***Stenotrophomonas maltophilia*** | + | – | – | | Elastase | Environmental, mucosal breaks, water, nosocomial | Pneumonia, bacteremia |
| ***Acinetobacter*** | + | – | – | | Capsule | Environmental, skin colonization, water, nosocomial | Respiratory, urinary catheter bacteremia |
| ***Burkholderia*** | | | | | | | |
| *B. mallei* | + | – | – | | | Contact with horses | Glanders |
| *B. pseudomallei* | + | – | – | | Lethal toxin, dermonecrotic toxin | Environmental in Southeast Asia and tropical regions | Melioidosis |
| *B. cepacia* | + | – | – | Pili | Elastase | Environmental, mucosal breaks, water, nosocomial | Wounds, pneumonia, cystic fibrosis |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Aeromonas*** | + | – | – | | Enterotoxin, cytotoxin | Environmental, fresh and salt water, leeches, intestinal flora | Wounds, diarrhea |
| ***Plesiomonas*** | + | – | – | | Enterotoxin | Water, seafood, soil | Diarrhea |
| ***Alkaligenes*** | + | – | – | | | Respiratory, intestinal flora | Blood, urine, wounds |
| ***Cardiobacterium*** | – | + | – | | | Nasopharyngeal, intestinal flora | Endocarditis |
| ***Chromobacterium*** | + | – | Violet | | | Water, soil, (tropical) | Cellulitis, bacteremia |
| ***Flavobacterium*** | – | – | Yellow | | | Environmental, nosocomial | Meningitis |
| ***Eikenella*** | – | + | – | | | Respiratory flora | Oropharyngeal abscess, draining sinuses |
| ***Actinobacillus*** | – | + | – | | | Respiratory flora, animals | Endocarditis, periodontal disease |
| ***Moraxella*** | – | – | – | Pili | | Respiratory flora | Bronchitis, pneumonia |

## ADDITIONAL READING

Govan JRW, Deretic V. Microbial pathogenesis in cystic fibrosis: Mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev* 1996;60:539–574. This review nicely covers the two Gram-negative rods that complicate the lives of those with cystic fibrosis.

Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: Our worst nightmare? *Clin Infect Dis* 2002;34:634–640. A review that illustrates the enormous capabilities of *P. aeruginosa* to develop resistance quickly.

Van Delden C, Iglewski BH. Cell to cell signaling and *Pseudomonas aeruginosa* infections. *Emerg Infect Dis* 1998;4:551–560. The complex way *P. aeruginosa* deploys its virulence factors is concisely explained and well illustrated.

CHAPTER 24

# *Haemophilus* and *Bordetella*

Kenneth J. Ryan

*Haemophilus* and *Bordetella* are small, Gram-negative rods that tend to assume a coccobacillary shape. They are nonmotile, non–spore forming, with complex nutritional growth requirements for blood-containing media. Members of both genera contain species exclusively found in humans that cause respiratory infections. The major species are *Haemophilus influenzae,* a major cause of purulent meningitis and *Bordetella pertussis,* the cause of whooping cough.

## *Haemophilus*

*Haemophilus* are among the smallest of bacteria. The curved ends of the short (1.0 to 1.5 $\mu$m) bacilli makes many appear nearly round, hence the term coccobacilli. The cell wall has a structure similar to that of other Gram-negative bacteria. *H. influenzae* may have a polysaccharide capsule, but other species of *Haemophilus* are not encapsulated.

Tiny Gram-negative coccobacilli

The cultivation of *Haemophilus* species requires the use of culture media enriched with blood or blood products (Greek "haema," blood, and "philos," loving) for optimal growth. This requirement is attributable to the need for exogenous hematin and/or nicotinamide adenine dinucleotide (NAD). These growth factors, also termed X factor (hematin) and V factor (NAD), are both present in erythrocytes. In culture media, optimal concentrations of X and, particularly, V factors are not available to *Haemophilus* from blood unless the red blood cells are lysed by gentle heat (chocolate agar) or digested and added separately as a supplement. Although erythrocytes are the only convenient source of hematin, the V factor is present in a variety of biologic sources and is produced by some other bacteria and yeasts. These conditions are responsible for the "satellite phenomenon," in which the bacteria form colonies on blood agar only in the vicinity of a colony of *Staphylococcus* that is producing V factor. The several species of *Haemophilus* are defined by their requirement for X and/or V factor, $CO_2$ dependence, and other cultural characteristics (Table 24–1).

All require hematin (X) and/or NAD (V)

Chocolate agar has X and V factors

Satellite formation around colonies of *S. aureus* is based on V factor

Species of *Haemophilus* other than *H. influenzae* have the same biology described below for the nonencapsulated strains of *H. influenzae*. Most of these other *Haemophilus* species have been reported to cause systemic illness, including pneumonia, meningitis, arthritis, endocarditis, and soft tissue infections. Such cases are even more rare than those in which nonencapsulated *H. influenzae* cause invasive disease.

Species other than *H. influenzae* are similar

TABLE 24–1

**Features of *Haemophilus* and *Bordetella***

| Species | Type | Growth Requirement | Capsule | Adherence Factors | Toxins | Epidemiology | Disease |
|---|---|---|---|---|---|---|---|
| ***Haemophilus*** | | | | | | | |
| *H. influenzae* | a–f | X and V | Polysaccharide | Pili | — | Normal flora, respiratory droplet spread | Meningitis, epiglottitis, arthritis, sepsis, otitis media |
| *H. influenzae* | — | X and V | — | HMW proteins, pili | — | Normal flora, respiratory droplet spread | Otitis media, bronchitis, sinusitis |
| *H. ducreyi* | — | X | — | Pili | Cytolethal distending toxin | Sexual contact | Chancroid |
| Other species[a] | — | X or V | — | — | — | Normal flora | Bronchitis |
| ***Bordetella*** | | | | | | | |
| *B. pertussis* | — | Nicotinamide[b] | — | Fha, PT, pertactin | PT, adenylate cyclase, tracheal cytotoxin | Strict pathogen, respiratory droplet spread | Whooping cough |
| *B. parapertussis* | — | Nicotinamide | — | | — | Presumed similar to *B. pertussis* | Rhinitis, cough |
| *B. bronchiseptica* | — | Nicotinamide | — | | — | Dogs, rabbits | Rhinitis, cough |

*Abbreviations:* X factor, hematin; V factor, nicotinamide adenine dinucleotide (NAD); HMW, high-molecular-weight proteins (HMW1, HMW2); Fha, filamentous hemagglutinin; PT, pertussis toxin.

[a] *H. parainfluenzae, H. aphrophilus, H. hemolyticus.*

[b] Also requires additives to neutralize toxicity in standard media.

# *Haemophilus influenzae*

## BACTERIOLOGY

Six serotypes are based on capsular polysaccharide

Hib capsule is PRP

Nonencapsulated strains are less virulent

*Haemophilus* that meet the species requirements for *H. influenzae* may or may not have a capsule. Those that do are divided into six serotypes, designated a to f, based on the capsular polysaccharide antigen. The type b capsule is made up of a polymer of ribose, ribitol, and phosphate, called **polyribitol phosphate (PRP)**. These surface polysaccharides are strongly associated with virulence, particularly *H. influenzae* type b (Hib). The nonencapsulated, and thus nontypable, *H. influenzae* can be classified by a number of typing schemes based on outer membrane proteins and other factors. These protein systems can also be applied to capsulated *H. influenzae* but have no particular association with virulence.

# *H. influenzae* DISEASE

CLINICAL CAPSULE

Hib produces acute, life-threatening infections of the central nervous system, epiglottis, and soft tissues, primarily in children. Disease begins with fever and lethargy, and in the case of acute meningitis, can progress to coma and death in less than 1 day. In affluent countries, Hib disease has been controlled by immunization. *H. influenzae* also produces common but less fulminant infections of the bronchi, respiratory sinuses, and middle ear. The latter are usually associated with nonencapsulated strains.

## EPIDEMIOLOGY

*H. influenzae* can be found in the normal nasopharyngeal flora of 20 to 80% of healthy persons, depending on age, season, and other factors. Most of these are nonencapsulated, but capsulated strains, including Hib, are not rare. Prior to the introduction of effective vaccines, approximately 1 in every 200 children developed invasive Hib disease by the age of 5 years. Meningitis is the most common form and most often attacks those under 2 years of age. Cases of epiglottitis and pneumonia tend to peak in the 2- to 5-year age range. Over 90% of these cases are due to the single serotype, Hib.

Nasopharyngeal colonization is common

Meningitis develops in children under 2 years of age

By the end of the first decade of universal immunization with the Hib protein conjugate vaccine in the United States (see Prevention), invasive disease rates have already declined by 99%. Now, invasive disease strikes only 1 in 100,000 children, and most of these cases are not caused by the type b serotype. Similar results have been seen in other countries, but Hib disease continues in those unable to afford the vaccine. Under the direction of the World Health Organization, government and philanthropic efforts are now underway to make this vaccine available to all children throughout the world.

Immunization has dramatically reduced disease

Countries that cannot afford vaccine are targeted

At one time *H. influenzae*–caused meningitis was believed to be an isolated endogenous infection, but reports of outbreaks in closed populations and careful epidemiologic studies of secondary spread in families have changed this view. The risk of serious infection for unimmunized children under 4 years of age living with an index case is more than 500-fold that for nonexposed children. This risk indicates a need for prophylaxis for contacts in the susceptible age group. Rifampin is currently recommended for this purpose.

Person-to person spread is blocked by vaccine or prophylaxis

## PATHOGENESIS

### Invasive Disease

For unknown reasons, *H. influenzae* strains commonly found in the normal flora of the nasopharynx occasionally invade into deeper tissues. Bacteremia then leads to spread to the central nervous system and metastatic infections at distant sites such as bones and joints. These events seem to take place within a short period (<3 days) after an encounter with a new virulent strain. Systemic spread is typical only for capsulated *H. influenzae* strains, and over 90% of invasive strains are type b. Even among Hib strains there are distinct clones, which account for about 80% of all invasive disease worldwide, and other clones, which are rarely associated with invasion.

Only capsulated strains are invasive

Limited number of Hib clones account for disease

The pathogenic mechanisms involved in Hib invasiveness remain to be fully understood. Attachment to respiratory epithelial cells is mediated by pili and other adhesins. There is some evidence to suggest that this is a complex regulatory cascade, coordinating capsular biosynthesis and adherence factors that act cooperatively in establishing the microbe within susceptible hosts. *H. influenzae* can be seen to invade between the cells of the respiratory epithelium, and for a time resides between and below them. Once past the mucosal barrier, the antiphagocytic capsule confers resistance to opsonophagocytosis in the same manner as it does with other encapsulated bacteria (*Streptococcus pneumoniae, Neisseria meningitidis*). Endotoxin in the cell wall is toxic to ciliated respiratory cells, but

Pili and other adhesins bind to epithelial cells

Invasion goes between cells

Capsule prevents phagocytosis

endotoxemia is not a prominent feature of *Haemophilus* infection to the extent that it is with *N. meningitidis. H. influenzae* produces no known exotoxins.

### Localized Disease

Bacterial trapped in middle ear, sinuses, and bronchi produce localized infections

Most are noncapsulated strains

Adherence is related to surface proteins

Nonencapsulated *H. influenzae* produce disease under circumstances in which they are entrapped at a luminal site adjacent to the normal respiratory flora such as the middle ear, sinuses, or bronchi. This is usually associated with some compromise of normal clearing mechanisms, which is caused by a viral infection or structural damage. Consistent with their relative prevalence in the respiratory tract, nontypeable organisms account for more than 90% of localized *H. influenzae* disease, particularly otitis media, sinusitis, and exacerbations of chronic bronchitis. Nonencapsulated *H. influenzae* may have pili capable of promoting attachment to host cells, but they are relatively uncommon. However, a family of nonpilus, surface-exposed, high-molecular-weight proteins (eg, HMW1, HMW2) has been identified in nonencapsulated strains and have not been found in capsulated strains. These proteins also mediate adherence to epithelial cells, and some of them show homology with the filamentous hemagglutinin that plays an essential role in adherence of *Bordetella pertussis* to ciliated epithelial cells (see below).

## IMMUNITY

Anticapsular antibody is bactericidal and protective

Hib infections occur at ages when antibody is absent

Immunity to Hib infections has long been associated with the presence of anticapsular (PRP) antibodies, which are bactericidal in the presence of complement. The infant is usually protected by passively acquired maternal antibody for the first few months of life. Thereafter the presence of actively acquired antibody increases with age; it is present in the serum of most children by 10 years of age. The peak incidence of Hib infections in unimmunized populations is 6 to 18 months of age, when serum antibody is least likely to be present. This inverse relationship between infection and serum antibody is similar to that for *N. meningitidis* (see Fig 20–2). The major difference is that substantial immunity is provided by antibody directed against a single type (Hib) rather than the multiple immunotypes of other bacteria. Thus, systemic *H. influenzae* infections (meningitis, epiglottitis, cellulitis) are rare in adults. When such infections develop, the immunologic deficit is the same as that with meningococci—lack of circulating antibody.

T cell–independent response to PRP is poor at $<$ 18 months

Protein conjugate vaccine elicits T-cell response in infants

Like many polysaccharides, Hib PRP behaves as a T cell–independent antigen. B cells mount the primary response without significant involvement of helper T cells. Antibody responses from immunization with PRP are variable and typically poor at less than 18 months of age. Significant secondary responses from boosters are not elicited. Conjugation of PRP to protein dramatically improves the immunogenicity by eliciting the T-cell responses typical of protein antigens while preserving the specificity for PRP, even in infants.

# *H. influenzae* DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

Of the major acute Hib infections, meningitis accounts for just over 50% of cases. The remaining are distributed among pneumonia, epiglottitis, septicemia, cellulitis, and septic arthritis. Localized infections can be caused by capsulated strains including Hib, but most are noncapsulated *H. influenzae.*

### Meningitis

Acute purulent meningitis may follow sinusitis or otitis media

Hib meningitis follows the same pattern as other causes of acute purulent bacterial meningitis (see Chapter 67). Meningitis is often preceded by signs and symptoms of an upper respiratory infection, such as pharyngitis, sinusitis or otitis media. Whether these represent a predisposing viral infection or early invasion by the organism is not known.

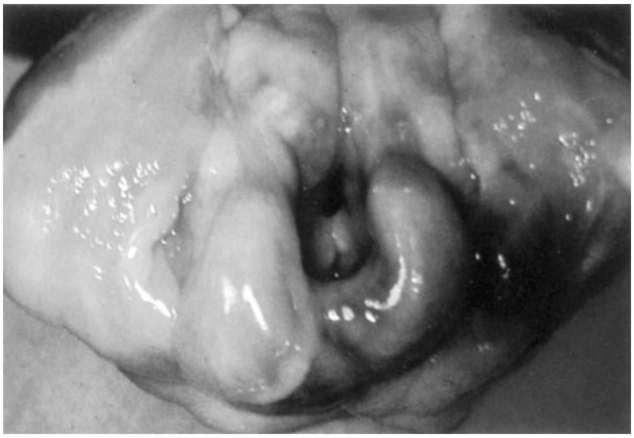

FIGURE 24–1
The swollen epiglottis characteristic of *Haemophilus influenzae* acute epiglottitis. (*Reproduced with permission from Connor DH, Chandler FW, Schwartz DQA, Manz HJ, Lack EE (eds).* Pathology of Infectious Diseases, *vol. 1. Stamford, CT: Appleton & Lange; 1997.*)

Just as often, meningitis is preceded by vague malaise, lethargy, irritability, and fever. Mortality is 3 to 6% despite appropriate therapy, and roughly one third of all survivors have significant neurologic sequelae.

Mortality and neurologic sequelae are significant

## Acute Epiglottitis

Acute epiglottitis is a dramatic infection in which the inflamed epiglottis and surrounding tissues obstruct the airway. Hib is one of a number of causes. The onset is sudden, with fever, sore throat, hoarseness, an often muffled cough, and rapid progression to severe prostration within 24 hours. Affected children have air hunger, inspiratory stridor, and retraction of the soft parts of the chest with each inspiration. The hallmark of the disease is an inflamed, swollen, cherry-red epiglottis that protrudes into the airway (Fig 24–1) and can be visualized on lateral x-rays. As with meningitis, this infection is treated as a medical emergency, with prime emphasis on antimicrobics and maintenance of an airway (tracheostomy or endotracheal intubation). Manipulations, including routine examination or attempting to take a throat swab, can trigger a fatal laryngospasm and acute obstruction.

Cherry-red, swollen epiglottis, and stridor are hallmarks

Airway maintenance is needed

## Cellulitis and Arthritis

A tender, reddish-blue swelling in the cheek or periorbital areas is the usual presentation of Hib cellulitis. Fever and a moderately toxic state are usually present, and the infection may follow an upper respiratory infection or otitis media. Joint infection begins with fever, irritability, and local signs of inflammation, often in a single large joint. *Haemophilus* arthritis is occasionally the cause of a more subtle set of findings, in which fever occurs without clear clinical evidence of joint involvement. Bacteremia is often present in both cellulitis and arthritis.

Cellulitis is usually facial

Large joints are involved

## Other Infections

*H. influenzae* is an important cause of conjuctivitis, otitis media, and acute and chronic sinusitis. It is also one of several common respiratory organisms that can cause and exacerbate chronic bronchitis. Most of these infections are caused by nonencapsulated strains and usually remain localized without bacteremia. Disease may be acute or chronic, depending on the anatomic site and underlying pathology. For example, otitis media is acute and painful because of the small, closed space involved, but after antimicrobic therapy and reopening of the eustachian tube, the condition usually clears without sequelae. The association of *H. influenzae* with chronic bronchitis is more complex. There is evidence that *H. influenzae* and other bacteria play a role in inflammatory exacerbations, but a unique cause-and-effect relationship has been difficult to prove. The underlying cause of the bronchitis is usually related to chronic damage resulting from factors such as smoking. *Haemophilus* pneumonia may be caused by either encapsulated or nonencapsulated organisms. Encapsulated strains have been observed to produce a disease much like

Nonencapsulated strains are common in otitis media, sinusitis, and bronchitis

Pneumonia is linked to underlying damage pneumococcal pneumonia; however, unencapsulated strains may also produce pneumonia, particularly in patients with chronic bronchitis.

## DIAGNOSIS

Blood cultures are useful in systemic infections

Demonstrating X and V requirement defines species

The combination of clinical findings and a typical Gram smear is usually sufficient to make a presumptive diagnosis of *Haemophilus* infection. The tiny cells are usually of uniform shape except in cerebrospinal fluid, where some may be elongated to several times their usual length. The diagnosis must be confirmed by isolation of the organism from the site of infection or from the blood. Blood cultures are particularly useful in systemic *H. influenzae* infections, because it is often difficult to obtain an adequate specimen directly from the site of infection. Bacteriologically, small coccobacillary Gram-negative rods that grow on chocolate agar but not blood agar strongly suggest *Haemophilus.* Confirmation and speciation depends on demonstration of the requirement for X and V factors and/or biochemical tests. Serotyping is unnecessary for clinical purposes but important in epidemiologic and vaccine studies.

## TREATMENT

Ampicillin-resistant strains produce $\beta$-lactamase

Third-generation cephalosporin is initial treatment

*H. influenzae* is often susceptible in vitro to ampicillin and amoxicillin, and usually susceptible to the newer cephalosporins, tetracycline, aminoglycosides, and sulfonamides. It is less susceptible to other penicillins and to erythromycin. Since the 1970s, the therapy of systemic infections has been complicated by the emergence of strains that produce a plasmid-mediated $\beta$-lactamase identical to that found in *Escherichia coli.* The frequency of resistant strains varies between 5 and 50% in different geographic areas. Ampicillin-resistant strains that do not produce $\beta$-lactamase also occur but are less common. Current practice is to start empiric therapy with a third-generation cephalosporin (eg, ceftriaxone, cefotaxime), which can be changed to ampicillin if susceptibility tests indicate that the infecting strain is susceptible.

## PREVENTION

PRP vaccine missed peak age of disease

PRP conjugated to bacterial proteins stimulates T cells

Purified PRP vaccines became available in 1985; however, due to the typically poor immune response of infants to polysaccharide antigens, their use was limited to children 24 months of age and older. Because immunization at this age misses the group most susceptible to Hib invasive disease, a new vaccine strategy was needed that included improved stimulation of T cell–dependent immune responses in infants. To achieve this, three PRP-protein conjugate vaccines were developed using proteins derived from *Corynebacterium diphtheriae* (toxoid, CRM 197) or *N. meningitidis* (outer membrane protein). The first PRP–protein conjugate vaccines were licensed in 1989, and by late 1990, they were recommended for universal immunization beginning at 2 months of age. As illustrated in Figure 24–2, the impact has been dramatic (see Epidemiology). So far

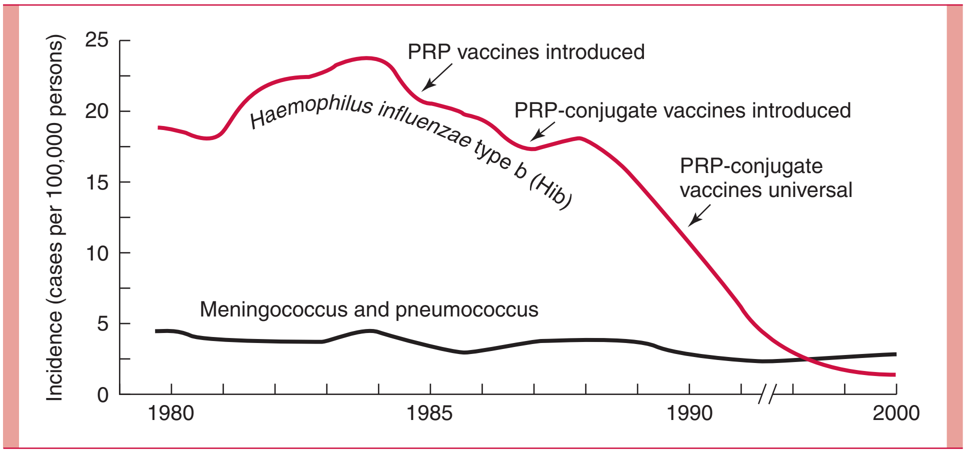


FIGURE 24–2
The recent decline in *Haemophilus influenzae* type b (Hib) meningitis and its association with the introduction of new vaccines.

this 99% reduction in what was the most common cause of childhood meningitis has not been accompanied by an increase in disease by either the non–type b strains or other causes of meningitis. An unexpected concomitant finding has been a dramatic drop in *H. influenzae* colonization rates in immunized populations.

Dramatic reduction in Hib disease has been sustained

# *Haemophilus ducreyi*

*H. ducreyi* causes chancroid, a common cause of genital ulcer in Africa, Southeast Asia, India, and Latin America. Occasional outbreaks in North America have most often been associated with the exchange of sex for drugs or money. The typical lesion is a tender papule on the genitalia that develops into a painful ulcer with sharp margins. Satellite lesions may develop by autoinfection, and regional lymphadenitis is common. The incubation period is usually short (2 to 5 days). The lack of induration around the ulcer has caused the primary lesion to be called "soft chancre" to distinguish it from the primary syphilitic chancre, which is typically indurated and painless. The presence of open genital sores due to *H. ducreyi* enhances the risk of transmission of HIV either by providing a portal of entry or by the recruitment of CD4+ cells to the site. This may contribute to the heterosexual spread of acquired immunodeficiency syndrome (AIDS) on the African continent, where chancroid is common. Candidate *H. ducreyi* virulence factors include adhesive pili, resistance to phagocytosis, and complement-mediated killing. A seeming lack of immunity may be due to the action of a toxin (cytolethal distending toxin) on T cells.

Soft chancre is a genital ulcer with satellite lesions

May contribute to spread of AIDS in Africa

The specific diagnosis of *H. ducreyi* infection is difficult. Although the organism grows on chocolate agar, it does so slowly and other organisms present in the genital flora are apt to overgrow the plates. A special medium incorporates vancomycin as a selective agent, but few laboratories in the United States have it on hand. Chancroid is effectively treated with azithromycin, ceftriaxone, or erythromycin.

Culture is difficult

# BORDETELLA

The genus *Bordetella* contains seven species. *B. pertussis* is by far the most important because it is the cause of classic pertussis (whooping cough). Nucleic acid homology and other analyses indicate that *B. parapertussis* and *B. bronchiseptica* are close enough to *B. pertussis* to be considered variants of the same species. *B. parapertussis* occasionally causes a disease similar to, but milder than, pertussis. This is probably because it does not produce pertussis toxin even though it has a silent copy of the toxin gene. The remainder of this section will focus solely on *B. pertussis.*

Species similar to *B. pertussis* do not cause classical whooping cough

# *Bordetella pertussis*

## BACTERIOLOGY

### GROWTH AND STRUCTURE

*B. pertussis* is a tiny (0.5 to 1.0 μm), Gram-negative coccobacillus morphologically much like *Haemophilus*. Growth requires a special medium supplemented with **nicotinamide** and other additives such as charcoal, which is thought to neutralize the effect of inhibitory

Coccobacilli are similar to *Haemophilus*

Nicotinamide required for slow growth

compounds present in standard bacteriologic media. Under the best conditions, growth is still slow, requiring 3 to 7 days for isolation. The organism is also very susceptible to environmental changes and survives only briefly outside the human respiratory tract.

Fha binds amino acid sequences found in host cells

Pili and pertactin are adhesins

The cell wall has the structure typical of Gram-negative bacteria, although the outer membrane lipopolysaccharide differs significantly in structure and biologic activity from that of the Enterobacteriaceae. The surface exhibits a rod-like protein called the **filamentous hemagglutinin (Fha)** because of its ability to bind to and agglutinate erythrocytes. Fha has strong adherence qualities, based on domains in its structure that interact with an amino acid sequence (arginine, glycine, aspartic acid) present in host integrins, epithelial cells, and macrophages. The organism surface also contains surface **pili** and the outer membrane includes a protein called **pertactin.**

## EXTRACELLULAR PRODUCTS

### Pertussis Toxin

A-B toxin ADP-ribosylates G protein

Adenylate cyclase and cell regulation are disrupted

**Pertussis toxin (PT)** is the major virulence factor of *B. pertussis*. It is an A-B toxin produced from a single operon as an enzymatic subunit and five distinct binding subunits that are assembled into the complete toxin on the bacterial surface. The binding subunits mediate attachment of the toxin to carbohydrate moieties on the host cell surface. The enzymatic subunit is then internalized and ADP-ribosylates a G-protein that affects adenylate cyclase activity. Unlike cholera toxin, which in essence keeps cyclase activity "turned on," pertussis toxin freezes the opposite side of the regulatory circuit and cripples the capacity of the host cell to inactivate cyclase activity. Other intracellular effector pathways are also disrupted by the G-protein modification. The binding subunits have a biologic effect on lymphocytes and other cells independent of the enzymatic function of the toxin.

### Other Toxins

Bacterial adenylate cyclase disrupts immune cell function

Peptidoglycan fragments injure tracheal cells

Another potent toxin, an invasive **adenylate cyclase,** enters host cells and catalyzes the conversion of host cell ATP to cyclic AMP at levels far above what can be achieved by normal mechanisms. This enzyme is hemolytic and interferes with cellular signaling, chemotaxis, superoxide generation, and microbicidal function of immune effector cells, including polymorphonuclear leukocytes and monocytes. It can also induce programmed cell death (apoptosis). Remarkably, after the adenylate cyclase enters the cell, it requires activation by calmodulin, a eukaryotic $Ca^{2+}$-binding protein. Such activation of a bacterial enzyme by an intracellular mammalian protein is unusual, but is also seen with another bacterial adenylate cyclase, anthrax toxin (see Chapter 18). **Tracheal cytotoxin** is essentially fragments of cell-wall peptidoglycan (1,6-anhydromuramic acid-*N*-acetylglucosamine-tetrapeptide). The fragments are released by multiplying bacterial cells and cause the death of ciliated tracheal cells. This cytotoxin is similar, if not identical to, one produced by *Neisseria gonorrhoeae* (see Chapter 20).

# PERTUSSIS (WHOOPING COUGH)

CLINICAL CAPSULE

Pertussis is a prolonged illness caused by toxins produced by *B. pertussis* bacteria attached to the cilia of respiratory epithelial cells. It progresses in stages over many weeks beginning with a rhinorrhea (runny nose) that evolves into a persistent cough. The name "whooping cough" comes from children who exhibit an inspiratory "whoop" following an exhausting series of paroxysmal coughs.

## EPIDEMIOLOGY

*B. pertussis* is spread by airborne droplet nuclei produced by patients in the early stages of illness. It is highly contagious, infecting 80 to 100% of exposed susceptible persons. Secondary spread in families, schools, and hospitals is rapid. Sporadic epidemics occur, but there is no strong seasonal pattern. *B. pertussis* is not found in animals and survives poorly in the environment. Asymptomatic carriers are very rarely found. However, infections in previously immunized adults have become an increasingly important reservoir, because the mild symptoms are often not recognized as any more than a "bad cold." Unwitting adults have served as the source for outbreaks in highly susceptible populations, such as infants in a newborn nursery. Mortality remains the highest in infants, with over 70% of fatal cases occurring in children under 1 year of age.

Highly contagious and spread by airborne droplet nuclei

Atypical, unrecognized disease in adults facilitates spread

After the introduction of immunization in the 1940s, the incidence of pertussis in the United States dropped from over 250,000 cases a year to well below one case per 100,000 population. Since the 1980s, a slow rise, augmented by epidemics every 3 to 4 years, has resulted. The 7796 cases reported in 1996 was a 30-year high. The greatest increase has been in the 10- to 20-year-age group. In general, pertussis has increased when immunization rates have fallen largely due to concerns about vaccine reactions. For example, when childhood immunization rates in England fell below 50% in 1981, pertussis cases rose dramatically. There were 47,000 cases in the first 9 months of 1982 alone.

Immunization reduces disease

Outbreaks correlate with incomplete immunization

## PATHOGENESIS

*B. pertussis* is a strict human pathogen. When introduced into the respiratory tract, the organism has a remarkable tropism for ciliated bronchial epithelium attaching to the cilia themselves. This adherence is mediated by Fha, pili, pertactin, and the binding subunits of PT. Once attached, the bacteria immobilize the cilia and begin a sequence in which the ciliated cells are progressively destroyed and extruded from the epithelial border (Fig 24–3). This local injury is caused primarily by the action of the tracheal cytotoxin. This produces an epithelium devoid of the ciliary blanket, which moves foreign matter away from the lower airways. Persistent coughing is the clinical correlate of this deficit. Although considerable local inflammation and exudate are produced in the bronchi, *B. pertussis* does not directly invade the cells of the respiratory tract or spread to deeper tissue sites.

Only infects humans

Attachment to cilia provides site for toxin production

Mucosa becomes devoid of ciliated cells

### Virulence Factors

In addition to the local effects on the bronchial epithelium, the virulence factors of *B. pertussis* contribute to the disease in many other ways. The combined action of PT and

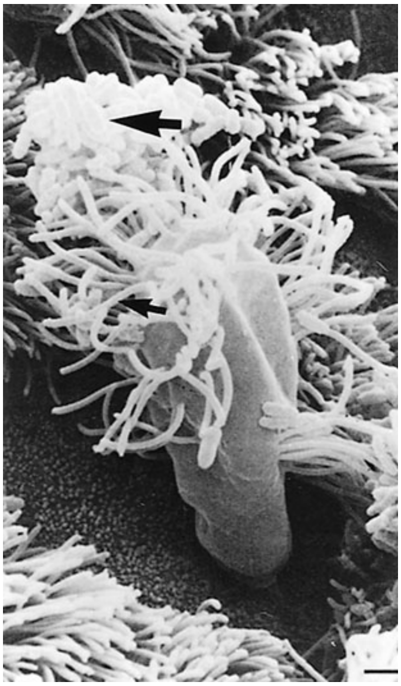

FIGURE 24–3
A tracheal organ culture 72 hours after infection with *Bordetella pertussis*. The organisms have attached to the cilia of some cells and killed them. These balloon-like cells with attached bacteria are extruded from the epithelium. The large arrow shows the *Bordetella* and the small arrow the cilia. Note the background of uninfected ciliated cells and denuded epithelium where nonciliated cells remain. (*Reproduced with permission from Muse KE, Collier AM, Baseman JB.* J Infect Dis *136:768–777. Figure 3, copyright 1977 by University of Chicago, publisher.*)

PT and adenylate cyclase attack immune cells

Absorbed PT acts on multiple cell types

adenylate cyclase on neutrophils, macrophages, and lymphocytes creates paralysis and even death of these crucial effector cells of the immune system. Many of the systemic manifestations of the disease such as lymphocytosis, histamine sensitization, and insulin secretion are due to the action of circulating PT absorbed at the primary infection site. The specific biologic effect depends on how disruption of G-protein regulation by PT is manifested by the host cell type the toxin reaches. Pertussis is the result of a well-orchestrated delivery by *B. pertussis* of toxic and adhesive factors to host cells at local and distant sites to produce a disease that persists for many weeks.

### Genetic Regulation of Pathogenicity

Multiple virulence genes respond to temperature and ionic changes

How *B. pertussis* deploys its repertoire of virulence genes is a model for the control of bacterial pathogenicity (Fig 24–4). *B. pertussis* regulates the synthesis of PT, the invasive adenylate cyclase, Fha, pili, and many other genes through genetic loci that control the expression of at least 20 unlinked chromosomal genes at the transcriptional level. Expression is modulated by changes in specific environmental parameters, including temperature.

Virulence genes are activated in two-compartment model

Environmental factors trigger Fha, PT, and adenylate cyclase when needed

Transcriptional regulation is controlled by a two-compartment system, which involves a regulatory protein that spans the bacterial membrane (BvgS) and an activator protein in the cytoplasm (BvgA). The membrane protein has a periplasmic domain that responds to temperature or ionic changes. When the temperature changes from 25°C to 37°C, it autophosphorylates and subsequently donates a phosphate group to the cytoplasmic protein, allowing it to bind to DNA recognition sequences in the chromosome. There it promotes the transcription of its own gene and those located in an operon containing at least the Fha and pilin structural genes. Experimentally, Fha, pilin, and BvgA mRNA are produced at this point, followed about 6 hours later by PT and adenylate cyclase. This delay is believed to be due to the time required for the production of a second BvgS directed activator which then turns on these genes.

Adherence factors precede injury products

Thus, the induction of virulence factors in *B. pertussis* is sequential, with adhesin expression (Fha and pili) preceding expression of factors involved in tissue injury. The finely honed responses of *B. pertussis* virulence factors to changes in temperature and ionic conditions presumably play a role in the pathogenesis of infection and help the organism adapt in a stepwise fashion to the diverse local conditions within the human respiratory tract.

## IMMUNITY

IgG to virulence factors does not produce long-term immunity

Although IgG antibodies are produced to PT, pili, and pertactin during the course of natural infection and by immunization, they are not long lasting and their role in immunity is not well understood. Naturally acquired immunity is not lifelong, although second attacks, when recognized, tend to be mild. The high susceptibility of newborns and infants before immunization may reflect a low level of antibody in adults and thus lack of passive transfer to the infant at birth.

# PERTUSSIS: CLINICAL ASPECTS

## MANIFESTATIONS

Catarrhal phase is most communicable

After an incubation period of 7 to 10 days, pertussis follows a prolonged course consisting of three overlapping stages: (1) catarrhal, (2) paroxysmal, and (3) convalescent. In the catarrhal stage, the primary feature is a profuse and mucoid rhinorrhea that persists for 1 to 2 weeks. Nonspecific findings such as malaise, fever, sneezing, and anorexia may also be present. The disease is most communicable at this stage, because large numbers of organisms are present in the nasopharynx and the mucoid secretions.

Paroxysmal coughing phase lasts for weeks

The appearance of a persistent cough marks the transition from the catarrhal to the paroxysmal coughing stage. At this time, episodes of paroxysmal coughing occur up to

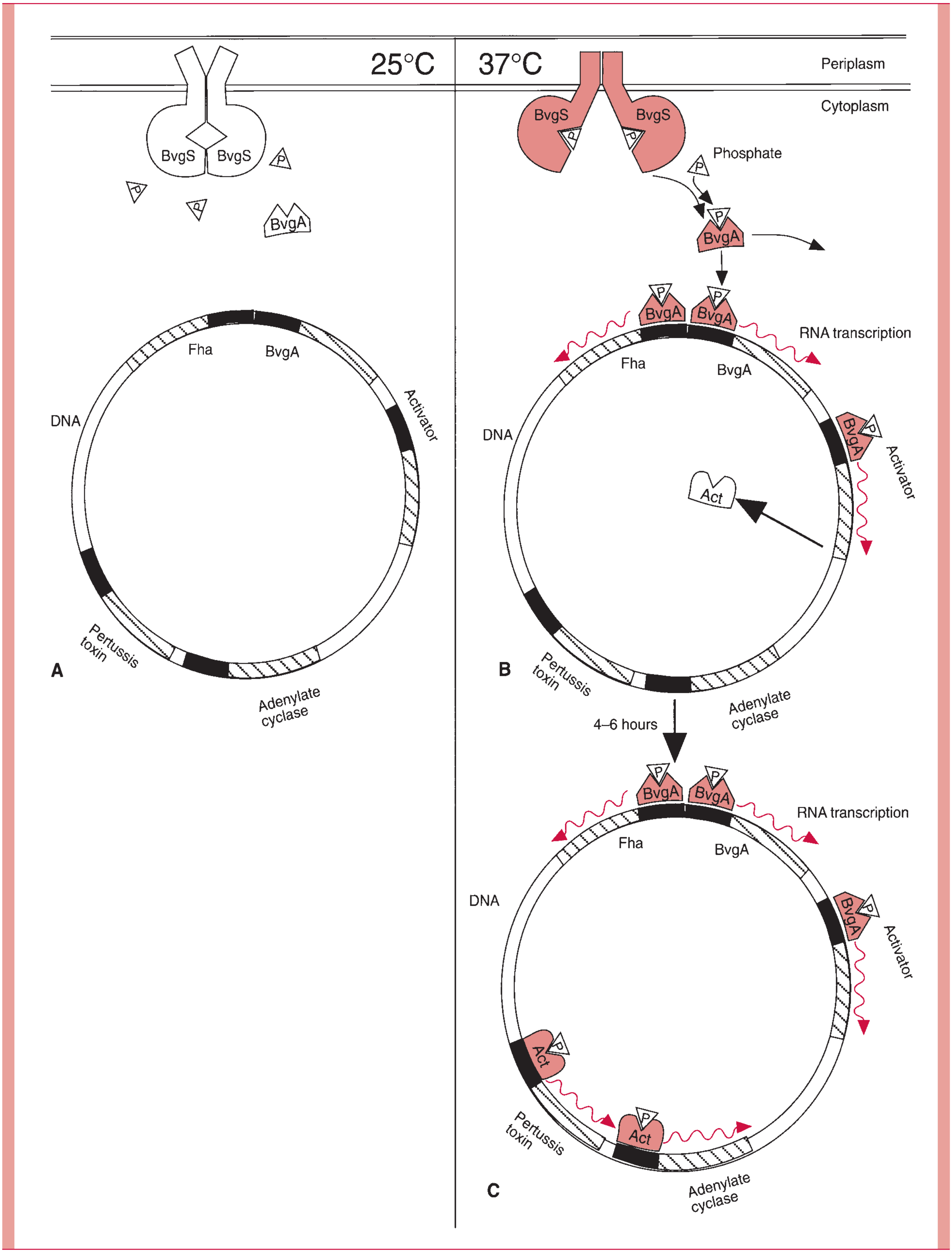


FIGURE 24–4

Regulation of *Bordetella pertussis* virulence factors. **A.** At 25°C the membrane-associated regulatory protein BvgS is inactive as are the genes for virulence factors filamentous hemagglutinin (Fha), pertussis toxin, and adenylate cyclase. **B.** At 37°C BvgS autophosphorylates and activates a cytoplasmic regulatory protein, BvgA, by phosphorylation. BvgA activates transcription of genes for production of BvgS, BvgA, Fha, and a postulated second regulator, Act. **C.** Hours later transcription of the pertussis toxin and adenylate cyclase is activated by Act. (*Adapted from Melton, AR, Weiss AA. Characterization of environmental regulators of* Bordetella pertussis. *Infect Immun 1993;61:807–815.*)

Inspiratory whoop and coughing may lead to apnea

Lymphocytosis is marked

50 times a day for 2 to 4 weeks. The characteristic inspiratory whoop follows a series of coughs as air is rapidly drawn through the narrowed glottis. Vomiting frequently follows the whoop. The combination of mucoid secretions, whooping cough, and vomiting produces a miserable, exhausted child barely able to breathe. Apnea may follow such episodes, particularly in infants. Marked lymphocytosis reaches its peak at this time, with absolute lymphocyte counts of up to 40,000/$mm^3$.

Convalescent phase is a gradual fading

During the 3- to 4-week convalescent stage, the frequency and severity of paroxysmal coughing and other features of the disease gradually fade. Partially immune persons and infants under 6 months of age may not show all the typical features of pertussis. Some evolution through the three stages is usually seen, but paroxysmal coughing and lymphocytosis may be absent.

Atelectasis and superinfection are major complications

The most common complication of pertussis is pneumonia caused by a superinfecting organism such as *S. pneumoniae*. Atelectasis is also common but may be recognized only by radiologic examination. Other complications, including convulsions and subconjunctival or cerebral bleeding, are related to the venous pressure effects of the paroxysmal coughing and the anoxia produced by inadequate ventilation and apneic spells.

## DIAGNOSIS

Nasopharyngeal swab is plated on charcoal blood agar

Organisms are often gone by later paroxysmal phase

A clinical diagnosis of pertussis is best confirmed by isolation of *B. pertussis* from nasopharyngeal secretions or swabs. Throat swabs are not suitable, because the cilia to which the organism attaches are not found there. Specimens collected early in the course of disease (during the catarrhal or early paroxysmal stage) provide the greatest chance of successful isolation. Unfortunately, the diagnosis is frequently not considered until paroxysmal coughing has been present for some time, and the number of organisms has decreased significantly. The nasopharyngeal specimens are plated onto a special charcoal blood agar medium made selective by the addition of a cephalosporin. This allows the slow-growing *B. pertussis* to be isolated in the presence of more rapidly growing members of the normal upper respiratory flora. The characteristic colonies appear after 3 to 7 days of incubation and look like tiny drops of mercury. Immunologic methods (agglutination, immuofluorescence) are required for specific identification.

DFA allows rapid diagnosis

A direct immunofluorescent antibody (DFA) technique has been successfully applied to nasopharyngeal smears for rapid diagnosis of pertussis. DFA is particularly helpful in pertussis because of the many days required for culture results. Because the sensitivity and specificity of DFA can vary with the quality of the reagents, these results should always be confirmed by culture, if possible. Serologic and molecular diagnostic methods have been developed but are not widely used for clinical diagnosis.

## TREATMENT

Erythromycin or clarithromycin are effective in catarrhal phase

Once the paroxysmal coughing stage has been reached, the treatment of pertussis is primarily supportive. Antimicrobial therapy is useful at earlier stages and for limiting spread to other susceptible individuals. Of a number of antimicrobics active in vitro against *B. pertussis,* erythromycin or clarithromycin are preferred because of their clinical effectiveness and relative lack of toxicity.

## PREVENTION

Whole cell vaccine was effective but had side effects

Acellular vaccines are purified preparations

Active immunization is the primary method of preventing pertussis. The original vaccine, which produced a 99% reduction in disease, was prepared from inactivated whole cell suspensions and given together with diphtheria and tetanus toxoids as DTP. The undoubted efficacy of this vaccine was colored by a high rate of side effects due to the crude nature of the whole cell preparation. These included local inflammation, fever and, rarely, febrile seizures. Although permanent neurologic sequelae were never convincingly linked to pertussis immunization, there were those who argued that the vaccine was worse than the disease. This led to the development of acellular vaccines, guided by knowledge of the virulence factors involved in the pathogenesis of pertussis. One type of vaccine is made by purification of virulence factors from whole cell preparations followed by formaldehyde inactivation where appropriate. Another vaccine strategy is the production of recombinant components, genetically engineered to be immunogenic but nontoxic.

The multiple acellular vaccines licensed in the United States have different combinations of virulence factors. All contain PT toxoid and Fha and some add pertactin or pili (vaccine manufacturers use the term fimbriae). The efficacy of these vaccines has now been established and all have dramatically lower frequencies of side effects. They have been combined with diphtheria and tetanus toxoids as DTaP replacing the whole cell DTP. This vaccine is now recommended for the full primary immunization (2, 4, and 6 months) and boosters (15–18 months, 4–6 years). Additional boosters are recommended every 10 years after the last dose.

Vaccines include PT, Fha, and other virulence factors

DTaP has replaced DTP

## ADDITIONAL READING

Bisgard KM, Kao A, Leake J, Strebel PM, Perkins BA, Wharton M. *Haemophilus influenzae* invasive disease in the United States, 1994–1995: Near disappearance of a vaccine-preventable childhood disease. *Emerg Infect Di*s 1998;4:229–237. This concise paper documents one of the most successful episodes in the history of immunization.

DeSerres G, Shadman R, Duval B, et al. Morbidity of pertussis in adolescents and adults. *J Infect Dis* 2000;182:174–179. A large group of patients was studied, showing that morbidity can be significant in age groups other than children, particularly in asthmatics, smokers, and older adults.

Fothergill LD, Wright J. Influenzal meningitis: The relation of age incidence to the bactericidal power of blood against the causal organism. *J Immunol* 1933;24:273–284. A classic study, the first to advance the currently accepted concepts of humoral immunity in *H. influenzae* disease.

Hewlett EL. Pertussis: Current concepts of pathogenesis and prevention. *Pediatr Infect Dis J* 1997;16:S78–S84. A concise summary of the role of *B. pertussis* virulence factors.

Muse KE, Collier AM, Baseman JB. Scanning electron microscopic study of hamster tracheal organ cultures infected with *Bordetella pertussis. J Infect Dis* 1977;136:768–777. The unique tropism of *B. pertussis* for ciliated cells and the subsequent destruction of those cells are shown experimentally and visually.

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CHAPTER 25

# *Mycoplasma* and *Ureaplasma*

W. LAWRENCE DREW

*Mycoplasma* and *Ureaplasma* are unique microbes in that they lack a cell wall. They are ubiquitous in nature as the smallest of free-living microorganisms. Numerous *Mycoplasma* species have been isolated from animals and humans, but only three species have been associated with human disease (Table 25–1). *Mycoplasma pneumoniae* is a lower respiratory tract pathogen. *Mycoplasma hominis* and *Ureaplasma urealyticum* cause genitourinary tract infections.

## MICROBIOLOGY

The organisms have diameters of about 0.2 to 0.3 mm, but they are highly plastic and pleomorphic and may appear as coccoid bodies, filaments, and large multinucleoid forms. They do not have a cell wall and are bounded only by a single triple-layered membrane (Fig 25–1) that, unlike other bacteria, contains sterols. The sterols are not synthesized by the organism but are acquired as essential components from the medium or tissue in which the organism is growing. Lacking a cell wall, *Mycoplasma* and *Ureaplasma* stain poorly or not at all with the usual bacterial stains. Their double-stranded DNA genome is small, probably because of lack of genes encoding a complex cell wall. *M. pneumoniae* is an aerobe, but most other species are facultatively anaerobic. All grow slowly in enriched liquid culture medium and on special *Mycoplasma* agar to produce minute colonies only after several days of incubation. The center of the *M. pneumoniae* colony grows into the agar and appears denser, giving the appearance of an inverted “fried egg.” Growth in culture is inhibited by specific antisera directed at the particular species. Colonies of *M. pneumoniae* bind red blood cells (RBCs) onto the surface of agar plate cultures (hemadsorption). This is due to binding by the mycoplasma to sialic acid–containing oligosaccharides present on the RBC surface.

No cell walls

Cell membrane contains sterols

Not stained well by common methods

Slow growth in specialized media

Hemadsorption is a feature of *M. pneumoniae*

TABLE 25–1

**Pathogenic *Mycoplasma* and *Ureaplasma* Species of Humans**

| Organism | Site | Prevalence | Disease |
|---|---|---|---|
| *M. pneumoniae* | Upper and lower respiratory tract | Common | Primary atypical pneumonia |
| *M. hominis* | Genitourinary tract | Common | Postpartum fever; pelvic inflammatory disease |
| *U. urealyticum* | Genitourinary tract | Very common | Nongonococcal urethritis |

## *MYCOPLASMA PNEUMONIAE*

### MYCOPLASMAL PNEUMONIA

CLINICAL CAPSULE

*M. pneumoniae* produces a common form of pneumonia, which tends to occur in any season and has a predilection for younger individuals. The illness is characterized by a nonproductive cough, fever, and headache, with radiologic and clinical evidence of scattered areas of pneumonia. The course is almost always benign, but improvement is accelerated by treatment with non–cell wall–active antimicrobials.

#### EPIDEMIOLOGY

Infecting dose is very low

Found worldwide most often in teenagers

*M. pneumoniae* accounts for approximately 10% of all cases of pneumonia. Infection is acquired by droplet spread. Experimental challenges indicate that the human infectious dose is very low, possibly less than 100 colony-forming units. Endemic infections with *M. pneumoniae* occur worldwide, but they are especially prominent in temperate climates. Epidemics at 4- to 6-year intervals have been noted in both civilian and military populations. The most common age for symptomatic *M. pneumoniae* infection is between 5 and 15 years, and the disease accounts for more than one third of all cases of pneumonia

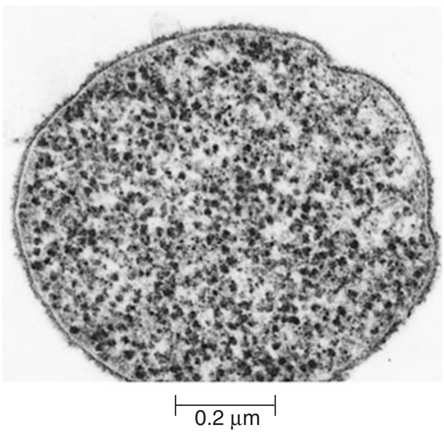


FIGURE 25–1
Electron micrograph of *Mycoplasma*. Note cytoplasmic membrane ribosomes and surface amorphous material with absence of cell wall. (*Courtesy of the late Dr. E. S. Boatman.*)

in teenagers, but is also seen in older persons. Infections in children less than 6 months of age are uncommon. The disease often appears as a sporadic, endemic illness in families or closed communities because its incubation period is relatively long (2 to 15 days) and because prolonged shedding in nasal secretions may cause infections to be spread over time. In families, attack rates in susceptible individuals approach 60%. Asymptomatic infections occur, but most studies have suggested that more than two thirds of infected cases develop some evidence of respiratory tract illness.

Outbreaks occur in families and closed communities

## PATHOGENESIS

*M. pneumoniae* infection involves the trachea, bronchi, bronchioles, and peribronchial tissues, and may extend to the alveoli and alveolar walls. Initially, the organism attaches to the cilia and microvilli of the cells lining the bronchial epithelium. This attachment is mediated by a surface mycoplasmal cytadhesin (P1) protein that binds to complex oligosaccharides containing sialic acid found in the apical regions of bronchial epithelial cells. The oligosaccharide receptors are chemically similar to the I antigen on the surface of erythrocytes and are not found on the nonciliated goblet cells or mucus, to which *M. pneumoniae* does not bind. The organisms interfere with ciliary action and initiate a process that leads to desquamation of the involved mucosa and a subsequent inflammatory reaction and exudate. The inflammatory response is at first most pronounced in the bronchial and peribronchial tissue and is composed of lymphocytes, plasma cells, and macrophages, which may infiltrate and thicken the walls of the bronchioles and alveoli. Organisms are shed in upper respiratory secretions for 2 to 8 days before the onset of symptoms, and shedding continues for as long as 14 weeks after infection.

Adherence to bronchial epithelial cells is mediated by P1 protein

Interferes with ciliary action and leads to desquamation

## IMMUNITY

Both local and systemic specific immune responses occur. Local IgA antibody is produced but disappears 2 to 4 weeks after the onset of the infection. Complement-fixing serum antibody titers reach a peak 2 to 4 weeks after infection and gradually disappear over 6 to 12 months. Nonspecific immune responses to the glycolipids of the outer membrane of the organism often also develop, which can be detrimental to the host. For example, cold hemagglutinins are IgM antibodies that react with the I antigen of human RBCs and are seen in about two thirds of symptomatic patients infected with *M. pneumoniae*.

Complement-fixing antibody titers peak at 2–4 weeks

Cold agglutinins are IgM

Immunity is not complete, and reinfection with *M. pneumoniae* is common. Clinical disease appears to be more severe in older than in younger children, which has led to the suggestion that many of the clinical manifestations of disease are the result of immune responses rather than invasion by the organism. High titers of cold agglutinations may be associated with hemolysis and Raynaud's phenomena. Antibodies may develop in response to an alteration of the I antigen by the organism or may represent cross-reacting antibodies.

Immunity is incomplete, and reinfection is common

# MYCOPLASMAL PNEUMONIA: CLINICAL ASPECTS

## MANIFESTATIONS

A mild tracheobronchitis with fever, cough, headache, and malaise is the most common syndrome associated with acute *M. pneumoniae* infection. The pneumonia is typically less severe than other bacterial pneumonias. It has been described as "walking" pneumonia, because most cases do not require hospitalization. The disease is of insidious onset, with fever, headache, and malaise for 2 to 4 days before the onset of respiratory symptoms. Pulmonary symptoms are generally limited to a non- or minimally productive cough. X-rays reveal a unilateral or patchy pneumonia, usually in a lower lobe, although multiple lobes are sometimes involved. Small pleural effusions are seen in up to 25% of cases.

"Walking" pneumonia has insidious onset

Cough is usual

Pharyngitis and otitis are also common

Pharyngitis with fever and sore throat may also occur. Nonpurulent otitis media or myringitis occurs concomitantly in approximately 15% of patients with *M. pneumoniae* pneumonitis. The presence of nonpurulent otitis media and lower respiratory illness in a teenager suggests *M. pneumoniae* infection.

## DIAGNOSIS

Diagnosis is usually serologic

Single high complement fixation or IgM-specific antibody titer supports diagnosis

Clinical diagnosis of *M. pneumoniae* infection may be difficult because the manifestations overlap with those of bacterial and viral infections. Gram-stained sputum usually shows some mononuclear cells, but, because it lacks a cell wall, *M. pneumoniae* is not seen. The absence of organisms, however, may help to suggest the etiology. The organism can be isolated from throat swabs or sputum of infected patients using special culture media and methods, but because of its slow growth, isolation usually requires incubation for a week or longer. Thus, serologic tests rather than cultures are more commonly used for specific diagnosis. A fourfold rise of serum antibody titer in acute and convalescent sera indicates *M. pneumoniae* infection. The most widely used serologic method is complement fixation. With the relatively long incubation period and insidious onset of the disease, many patients already have high antibody titers at the time they are first seen. In these situations, a single high titer, such as a complement fixation titer greater than 1:128 or IgM-specific antibody (measured by enzyme immunoassay or immunofluorescence), indicates recent or current infection, because these antibodies are generally of short duration.

Cold agglutinins are nonspecific but helpful

Because more than two thirds of patients with symptomatic lower respiratory *M. pneumoniae* infection develop high titers of cold hemagglutinins, their demonstration can be useful in some clinical situations. It must be remembered that cold hemagglutinins are nonspecific and have been observed in adenovirus infections, infectious mononucleosis, and some other illnesses. The test is simple, however, and can be performed rapidly in any clinical laboratory. Direct detection of the organism in respiratory secretions has been attempted using immunoassay methods, DNA hybridization, and the polymerase chain reaction. These methods are not yet available for routine diagnosis.

## TREATMENT

Erythromycin, tetracycline, clarithromycin, or azithromycin used in treatment

Erythromycin or tetracycline are the usual agents used for treatment of *M. pneumoniae* infections. They shorten the course of infection, although eradication from the nasopharynx may take much longer. Azithromycin and clarithromycin are comparable to erythromycin, but clindamycin is not effective. Most quinolones are also active.

# *Mycoplasma hominis*

Genitourinary inhabitant

Grows rapidly on specialized agar

Association with postpartum fever

*M. hominis* is a common inhabitant of the genitourinary tract. Although some strains grow on ordinary blood agar as nonhemolytic pinpoint colonies, the organism is best detected on *Mycoplasma* agar, on which it grows rapidly. *M. hominis* and *Ureaplasma* can be differentiated by demonstrating arginine breakdown by the former and urease activity by the latter. At least seven antigenic variants of *M. hominis* have been described. To date, the major clinical condition associated with *M. hominis* infection is postabortal or postpartum fever. *Mycoplasma hominis* is isolated from the blood of about 10% of women with this condition. Occasional infections of the central nervous system or joints also have been described, primarily in patients with antibody deficiency syndromes or premature infants.

Association with pelvic inflammatory disease

Resistant to erythromycin

The diseases appear to be self-limiting, although antibiotic therapy may decrease the duration of fever and hospitalization. Serologic studies and animal experiments have also indicated that pelvic inflammatory disease syndromes in women may be associated with *M. hominis* infection of the fallopian tubes. The organism is sensitive to tetracycline. In contrast to *U. urealyticum* and *M. pneumoniae, M. hominis* is resistant to erythromycin.

## *Ureaplasma urealyticum*

The genus *Ureaplasma* contains a single species, *U. urealyticum*, of which some 14 serotypes have been described. *Ureaplasma* is distinguished from *Mycoplasma* by its production of urease. On special *Ureaplasma* agar media, colonies are small and circular and grow downward into the agar. In liquid media containing urea and phenol red, growth of *Ureaplasma* results in production of ammonia from the urea, with a resultant increase in pH and a change in color of the indicator.

Urease production marks the species

### EPIDEMIOLOGY

The main reservoir of human strains of *U. urealyticum* is the genital tract of sexually active men and women; it is rarely found before puberty. Colonization, which probably results primarily from sexual contact, occurs in more than 80% of individuals who have had three or more sexual partners.

Most commonly acquired by sexual contact

### MANIFESTATIONS

Because of the high colonization rate, it has been difficult to associate specific illness with *Ureaplasma*; however, studies suggest that approximately one half of cases of nongonococcal, nonchlamydial urethritis in men may be caused by *U. urealyticum.* In women, *Ureaplasma* has been shown to cause chorioamnionitis and postpartum fever. The organism has been isolated from 10% of women with the latter syndrome.

Association with urethritis in men

### DIAGNOSIS AND TREATMENT

Men with nongonococcal urethritis should be treated since *Ureaplasma* infection may be involved. Tetracycline is the treatment of choice because it is also active against *Chlamydia*, but tetracycline-resistant strains of *Ureaplasma* have been reported that have been associated with recurrences of nongonococcal urethritis in men. In such cases, spectinomycin treatment or treatment with quinolone antimicrobics is also effective. Women with postpartum fever due to *U. urealyticum* may respond to tetracycline treatment.

Tetracyclines, spectinomycin, or quinolones are effective

## ADDITIONAL READING

Taylor-Robinson D. Infections due to species of mycoplasma and ureaplasma: An update. *Clin Infect Dis* 1996;23:671–684. Excellent review by one of the “fathers” of the field.

Taylor-Robinson D, et al. Antibiotic susceptibilities of mycoplasma and treatment of mycoplasma infections. *J Antimicrob Chemother* 1997;40:622–630. Reviews the currently available agents for treatment of mycoplasma infections.

Waris ME, Toikka P, Saarinen T, et al. Diagnosis of *Mycoplasma pneumoniae* pneumonia in children. *J Clin Microbiol* 1998;36:3155–3159. This paper examines the pros and cons of various diagnostic tests.

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CHAPTER 26

# Legionella

KENNETH J. RYAN

*Legionella* is a genus of Gram-negative bacilli that take their name from the American Legion convention where they were first discovered. The species designation of the prime human pathogen, *Legionella pneumophila,* reflects its propensity to cause pneumonia. *Legionella* species are widespread in the environment.

## BACTERIOLOGY

### MORPHOLOGY AND STRUCTURE

*Legionella pneumophila* is a thin (0.5 to 0.7 $\mu$m), pleomorphic, Gram-negative rod that may show elongated, filamentous forms up to 20 $\mu$m long. In clinical specimens, the organism stains poorly or not at all by Gram stain or the usual histologic stains; however, it can be demonstrated by certain silver impregnation methods (Dieterle stain) and by some simple stains that omit decolorization steps. Polar, subpolar, and lateral flagella may be present. Most species of *Legionella* are motile. Spores are not found.

Gram-negative rod that stains with difficulty

Structurally, *L. pneumophila* has features similar to those of Gram-negative bacteria with a typical outer membrane, thin peptidoglycan layer, and cytoplasmic membrane. The toxicity of *L. pneumophila* lipopolysaccharide (LPS) is significantly less than that of other Gram-negative bacteria such as *Neisseria* and the Enterobacteriaceae. This has been attributed to chemical makeup of the LPS side chains, which are a homopolymer of an unusual sugar (legionaminic acid), which renders the cell surface highly hydrophobic. It has been postulated that this hydrophobicity may promote adherence of bacterial cells to membranes or their concentration in aerosols.

LPS is less toxic than that of most Gram-negative species

Side chains are hydrophobic

### GROWTH AND CLASSIFICATION

*Legionella* species fail to grow on common enriched bacteriologic media such as blood agar. This is due to unusual requirements for certain amino acids (L-cysteine), ferric ions, and slightly acidic conditions (optimal pH 6.9). Even when these requirements are met, growth under aerobic conditions is slow requiring 2 to 5 days to produce colonies that have a distinctive surface resembling ground glass.

Growth requires iron and low pH

Due to the difficulty in growing *Legionella* there are few phenotypic properties to use in its classification. It is possible to directly demonstrate some enzymatic actions (catalase, oxidase, $\beta$-lactamase), but the other cultural and metabolic taxonomic tests used to

Few phenotypic properties are demonstrable

Classification is based on antigenic structure and DNA homology

Multiple *L. pneumophila* serogroups and *Legionella* species exist

classify other bacteria cannot be applied to *Legionella*. Thus, the classification depends largely on antigenic features, chemical analysis, and nucleic acid homology comparisons.

*L. pneumophila* has 14 serogroups and there are more than 30 other *Legionella* species (eg, *L. bozemanii, L. dumoffii, L. micdadei*). The original Philadelphia strain (serogroup 1) is still the most common and a limited number of *L. pneumophila* serogroups (1 to 4) account for 80 to 90% of cases. Not all of the non–*L. pneumophila* species have been isolated from human infections.

# LEGIONELLOSIS

**CLINICAL CAPSULE**

*Legionella* are inhaled into the lung from an aquatic source in the environment. Once there, they produce a destructive pneumonia marked by headache, fever, chills, dry cough, and chest pain. Although there may be multiple foci in both lungs and extension to the pleura, spread outside the respiratory tree is very rare.

## EPIDEMIOLOGY

1976 outbreak lead to discovery of new bacterium

Earlier outbreaks have been solved

The widely publicized outbreak of pneumonia among attendees of the 1976 American Legion convention in Philadelphia led to the isolation of a previously unrecognized infectious agent, *L. pneumophila*. The event was unique in medical history; for months the American public had entertained theories of its cause that ranged from sabotage to viroids, only to find that a Gram-negative rod that could not be stained or grown by the common methods was responsible. It was an outstanding example of the benefits of pursuing sound epidemiologic evidence until it is explained by equally sound microbiologic findings. We now know the disease had occurred for many years. Specific antibodies and organisms have been detected in material preserved from the 1950s, and a mysterious hospital outbreak in 1965 has been solved retrospectively.

Amoebas in fresh water habitat act as reservoir

Infections are associated with aerosols distributed by humidifying and cooling systems

In nature, *Legionella* species are ubiquitous in fresh water particularly in warm weather. In these sites they are also found as parasites of protozoa including numerous species of amoebae which appear to be the environmental reservoir. Transmission to humans is possible when the water supply of buildings becomes colonized and the system includes devices that create aerosols. Most outbreaks have occurred in or around large buildings such as hotels, factories, and hospitals involving cooling towers or some other part of the air-conditioning system. Some hospital outbreaks have implicated respiratory devices and potable water coming from parts of the hot water system such as faucets and shower heads. *Legionella* can persist in a water supply despite what appear to be adequate levels of chlorine particularly if the pipes contain abundant scale and/or dead end branches.

Person-to-person transmission or carriers are unknown

Disease rate among exposed is low

Person-to-person transmission has not been documented, and the organisms have not been isolated from healthy individuals. It is difficult to ascertain the overall incidence of *Legionella* infections; most information has been from outbreaks. Serologic surveys indicate that outbreaks constitute only a small part of the total cases, many of which currently go undetected. Estimates based on seroconversions suggest approximately 25,000 cases in the United States each year. Both serologic and environmental studies indicate that *Legionella* has low virulence for humans. The attack rate among those exposed is estimated at less than 5% and most serious cases are in immunocompromised persons.

## PATHOGENESIS

Strong tropism for the lung

Necrotizing multifocal pneumonia with intracellular bacteria

*L. pneumophila* is striking in its propensity to attack the lung, producing a necrotizing multifocal pneumonia. Microscopically, the process involves the alveoli and terminal bronchioles, with relative sparing of the larger bronchioles and bronchi (Fig 26–1). The inflammatory exudate contains fibrin, polymorphonuclear neutrophils (PMNs), macro-

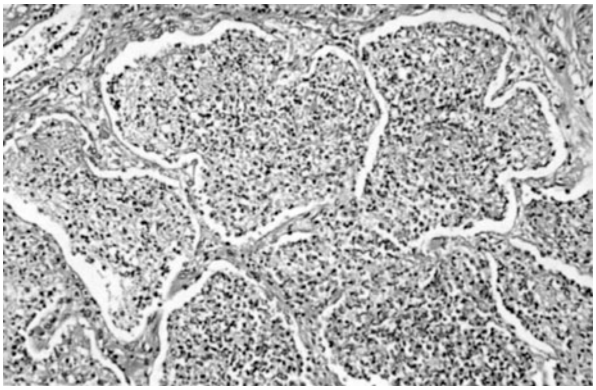

FIGURE 26–1
*Legionella* pneumonia. Note the filling of alveoli with exudate. Some of the alveolar septa are starting to degenerate. (*Reproduced with permission from Connor DH, Chandler FW, Schwartz DQA, Manz HJ, Lack EE (eds).* Pathology of Infectious Diseases, *vol. 1. Stamford, CT: Appleton & Lange; 1997.*)

phages, and erythrocytes. A striking feature is the preponderance of bacteria within phagocytes and the lytic destruction of inflammatory cells.

Facultative intracellular pathogen multiplies in alveolar macrophages

*L. pneumophila* is a facultative intracellular pathogen. Its pathogenicity depends on its ability to survive and multiply within cells of the monocyte–macrophage series. Inhaled *Legionella* bacteria reach the alveoli, where they enter alveolar macrophages utilizing

OMPs facilitate phagocyte entry to specialized vacuole

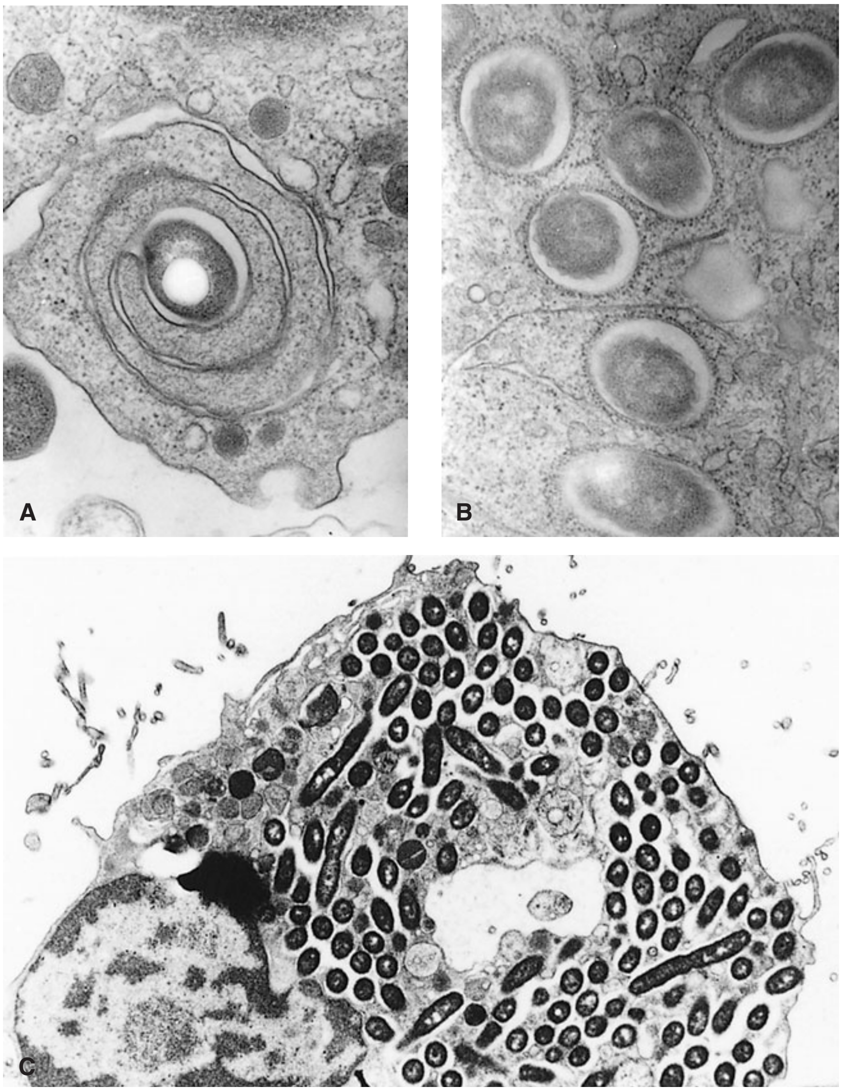


FIGURE 26–2
Multiplication of *Legionella pneumophila* in human macrophages. *Legionella pneumophila* enters the cell by coiling phagocytosis **(A)**, and the phagosome created is lined by ribosomes and mitochondria (**B**). The bacteria multiply within the macrophages to reach very high numbers **(C)**. (*Courtesy of Dr. Marcus Horwitz.*)

mechanisms involving multiple molecules. One outer membrane protein (OMP) binds C3, facilitating phagocyte recognition, and induces pores in the membrane of the macrophage. Another OMP called **macrophage invasion potentiator** (Mip) determines cell entry.

Secreted proteins block phagosomal fusion with lysosomes

Control of vesicular traffic creates replicative vacuole

Intracellular events are similar in amoeba

Inside the vacuole the bacteria continue to replicate by preventing phagosome-lysosome fusion and instead recruiting rough endoplasmic reticulum to the phagosome. The morphology of the replicative vacuole created is reflected in a process called **coiling phagocytosis** and is shown in Figure 26–2. *L. pneumophila* appears to accomplish this control of the phagocyte by use of a system that secretes proteins able to modulate host cell vesicle traffic. Other elements of the organism's intracellular success include its ability to extract iron from intracellular transferrin and a peptide toxin that inhibits activation of the oxidative killing mechanisms of PMNs. Thus, instead of being killed by the bactericidal mechanisms of phagocytes *L. pneumophila* multiplies freely. Death of cells is also related to induction of programmed cell death and formation of a pore-forming toxin. The progression of intracellular events in free-living amoebae is remarkably similar to that in human alveolar macrophages.

## IMMUNITY

Cytokine activated macrophages limit intracellular growth

Antibody is less important

Just as intracellular multiplication is the key to *L. pneumophila* virulence, its inhibition by cell-mediated mechanisms appears to be the most important aspect of immunity. Whether *L. pneumophila* is able to interfere with development of these responses is not known, but hypoexpression of major histocompatibility complex class I and II molecules has been observed in phagosomes containing the organisms. In immunocompetent persons cytokine-activated macrophages eventually inhibit intracellular multiplication and limit growth of *Legionella*. Most progressive cases of Legionnaires' disease are in immunocompromised patients. The role of humoral immunity appears to be less important. In the presence of activated cellular immune responses antibody may play an ancillary role through enhancement of phagocytosis. It is unknown whether humans who have had Legionnaires' disease are immune to reinfection and disease.

# LEGIONELLOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

Severe toxic pneumonia occurs in 5% of those exposed

Mortality is high among the immunocompromised

Legionnaires' disease is a severe toxic pneumonia that begins with myalgia and headache, followed by a rapidly rising fever. A dry cough may develop and later become productive, but sputum production is not a prominent feature. Chills, pleuritic chest pain, vomiting, diarrhea, confusion, and delirium may all be seen. Radiologically, patchy or interstitial infiltrates with a tendency to progress toward nodular consolidation are present unilaterally or bilaterally. Liver function tests often indicate some hepatic dysfunction. In the more serious cases the patient becomes progressively ill and toxic over the first 3 to 6 days, and the disease terminates in shock, respiratory failure, or both. The overall mortality is about 15%, but has been higher than 50% in some hospital outbreaks. It is particularly high in patients with serious underlying disease or suppression of cell-mediated immunity.

Pontiac fever may be hypersensitivity response

A less common form of disease called **Pontiac fever** (named for a 1968 Michigan outbreak), is a nonpneumonic illness with fever, myalgia, dry cough and a short incubation period (6 to 48 hours). Pontiac fever is a self-limiting illness and may represent a reaction to endotoxin or hypersensitivity to components of the *Legionella* or their protozoan hosts.

## DIAGNOSIS

High-quality specimens are needed

The best means of diagnosis is direct fluorescent antibody (DFA) smears combined with culture of infected tissues. For this purpose, a high-quality specimen such as lung aspirates, bronchoalveolar lavage, or biopsies are preferred, because the organism may not be found in sputum. Typically, the Gram smear shows no bacteria, but the organisms are demonstrated by DFA using *L. pneumophila*–specific conjugates. These conjugates utilize monoclonal antibodies, which bind to all serotypes of *L. pneumophila* but not the non–*L. pneumophila* species. DFA is rapid, but it is positive in only 25 to 50% of culture-proved cases.

DFA is rapid but only 50% sensitive

Cultures must be made on buffered charcoal yeast extract (BCYE) agar medium that meets the growth requirements of *Legionella*. BCYE contains amino acids, vitamins, L-cysteine, ferric pyrophosphate, and charcoal to adsorb toxic fatty acids. It is buffered optimally for *Legionella* growth (pH 6.9). The isolation of large Gram-negative rods on BCYE after 2 to 5 days that have failed to grow on routine media (blood agar, chocolate agar), is presumptive evidence for *Legionella*. Diagnosis is confirmed by DFA staining of bacterial smears prepared from the colonies. BCYE also allows isolation of species of *Legionella* other than *L. pneumophila.*

Culture on BCYE is required for isolation

Cultures will isolate other species

The diagnosis of legionellosis can also be established by polymerase chain reaction (PCR) amplification of a rRNA gene common to all *Legionella* species or detection of antigen by immunoassay of urine. The antigenuria test was originally limited to *L. pneumophila* serogroup 1 but has been recently expanded to all *L. pneumophila* serogroups. Demonstrating a significant rise in serum antibody is used primarily for retrospective diagnosis and in epidemiologic studies. Diagnostic procedures for legionellosis are likely to be available only in reference facilities and the laboratories of hospitals treating immunocompromised patients. Even here, DFA and culture remain the mainstay until the newer methods prove cost-effective.

PCR detects rRNA gene

Antigenuria is detected by immunoassay

## TREATMENT

The best information on antimicrobial therapy is still provided by the original Philadelphia outbreak. Because the etiology was completely obscure at the time, the cases were treated with many different regimens. Patients treated with erythromycin clearly did better than those given the penicillins, cephalosporins, or aminoglycosides. Subsequently, it was shown that most *Legionella* produce $\beta$-lactamases. In vitro susceptibility tests and animal studies have confirmed the activity of erythromycin and showed that tetracycline, rifampin, and the newer quinolones are also active. Although the other antimicrobics are sometimes used in combination, erythromycin and the newer macrolides (azithromycin, clarithromycin) remain the agents of choice.

Erythromycin is treatment of choice

Tetracycline, rifampin, and quinolones are alternatives

## PREVENTION

The prevention of legionellosis involves minimizing production of aerosols in public places from water that may be contaminated with *Legionella.* Although outbreaks connected with large buildings have received the most attention, cases have been traced to sources as common as the mists used in supermarkets to make the vegetables look shiny and fresh. Prevention is complicated by the fact that, compared with other environmental bacteria, *Legionella* bacteria are relatively resistant to chlorine and heat. They have been isolated from hot water tanks held at over 50°C. Methods for decontaminating water systems are still under evaluation. Some outbreaks have been aborted by hyperchlorination, by correcting malfunctions in water systems, or by temporarily elevating the system temperature above 70°C. The installation of silver and copper ionization systems similar to those used in large swimming pools has been effective as a last resort in hospitals plagued with recalcitrant nosocomial legionellosis.

Preventing *Legionella* aerosols is primary goal

Heat, hyperchlorination, and metal ions may be needed in institutions

## ADDITIONAL READING

Fraser DW, Tsai TR, Orenstein W, et al. Legionnaires' disease: Descriptions of an epidemic of pneumonia. *N Engl J Med* 1977;297:1189–1197.

McDade JE, Shepard CC, Fraser DW, et al. Legionnaires' disease: Isolation of a bacterium and demonstration of its role in other respiratory disease. *N Engl J Med*

1977;297:1197–1203. This study and the report by Fraser et al describe the 1976 outbreak at the Philadelphia American Legion convention and the methods that led to the discovery of the cause of this “new” disease. It makes good reading as an example of medical discovery and the requirements of proof.

Rohr U, Senger M, Selenka F, Turley R, Wilhelm M. Four years of experience with silver-copper ionization for control of *Legionella* in a German university hospital hot water plumbing system. *Clin Infect Dis* 1999;29:1507–1511. The paper illustrates the difficulty presented by the “colonization” of a hospital water supply by *Legionella.*

Stout JE, Yu VL. Legionellosis. *New Engl J Med* 1997;337:682–687. This concise review emphasizes clinical aspects.

CHAPTER 27

# Spirochetes

KENNETH J. RYAN

Spirochetes generally refer to bacteria with a spiral morphology ranging from loose coils to a rigid corkscrew shape. The three medically important genera include the cause of syphilis, the ancient scourge of sexual indiscretion, and Lyme disease, a newly discovered consequence of an innocent walk in the woods (Table 27–1).

## BACTERIOLOGY

### MORPHOLOGY AND STRUCTURE

The spiral morphology of spirochetes is produced by a flexible, peptidoglycan cell wall around which several axial fibrils are wound. These fibrils have the structure of flagella and are referred to as **endoflagella** (Fig 27–1). The cell wall and endoflagella are completely covered by an outer bilayered membrane similar to the outer membrane of other Gram-negative bacteria. In some species, a hyaluronic acid slime layer forms around the exterior of the organism and may contribute to its virulence. Spirochetes are motile, exhibiting rotation and flexion; this motility is believed to result from movement of the endoflagellar filaments, although the mechanism is not clear.

Spiral structure is wound around endoflagella

Motility includes rotation and flexion

Many spirochetes are difficult to see by routine microscopy. Although they are Gram-negative, many either take stains poorly or are too thin (0.15 $\mu$m or less) to fall within the resolving power of the light microscope. Only darkfield microscopy, immunofluorescence, or special staining techniques that effectively increase their diameter can demonstrate these spirochetes. Other spirochetes such as *Borrelia* are larger and readily visible in stained preparations, even routine blood smears.

Many are thin and take stains poorly

Darkfield demonstrates spirochetes

### GROWTH AND CLASSIFICATION

Parasitic spirochetes grow more slowly in vitro than most other disease-causing bacteria. Some species, including the causative agent of syphilis, have not been grown beyond a few generations in cell culture. Some are strict anaerobes, others require low concentrations of oxygen, and still others are aerobic. Compared to other bacterial groups the taxonomy of the spirochetes is underdeveloped. Because spirochetes are difficult to grow, they are difficult to study; thus, there are relatively few phenotypic properties on which to base a classification. The medically important genera *Treponema, Leptospira,* and *Borrelia* have been distinguished primarily by morphologic characters such as the nature of their spiral shape

Some have not been isolated in culture

May be aerobic or anaerobic

TABLE 27–1

## Features of Spirochetal Diseases

| | | | | Diagnosis | | | |
|---|---|---|---|---|---|---|---|
| Organism | Morphology | Transmission | Reservoir | Microscopy | Culture | Serology | Disease |
| *Treponema pallidum* | Corkscrew spirals | Sexual, transplacental, transfusion | Humans | Darkfield of chancre or secondary lesions | None | VDRL, RPR, FTA-ABS, MHA-TP | Syphilis |
| *Leptospira interrogans* | Close spirals, hooked ends | Ingestion of contaminated water | Rodents, cattle, dogs | Not recommended[a] | Rarely performed[b] | MAT | Fever, meningitis, hepatitis |
| *Borrelia recurrentis* | Loose spirals | Lice | Humans | Giemsa or Wright stain of blood smear | Rarely performed[c] | None | Relapsing fever |
| *Borrelia hermsii* | Loose spirals | Ticks[d] | Rodents | Giemsa or Wright stain of blood smear | Rarely performed[c] | None | Relapsing fever |
| *Borrelia burgdorferi* | Loose spirals | Ticks[e] | White-footed mice, other rodents, (deer)[f] | Not recommended[a] | Rarely performed[c] | EIA + Western blot | Lyme disease |

*Abbreviations:* FTA-ABS, fluorescent treponemal antibody; MAT, microagglutination test; MHA-TP, microhemagglutination test for *T. pallidum;* RPR, rapid plasma reagin; VDRL, Venereal Disease Research Laboratory.

[a] Organisms are small in number and rarely seen in clinical lesions.
[b] Culture of blood or urine in semisolid Fletcher's medium takes 1 to many weeks and is generally not available.
[c] Culture of blood in liquid Barbour–Stoener–Kelly medium takes 1 to many weeks and is generally not available.
[d] *Ornithodoros hermsi.*
[e] *Ixodes scapularis* in the eastern and central United States, *I. pacificus* in the western United States.
[f] Transmitting ticks mature on deer that are not actually a reservoir.

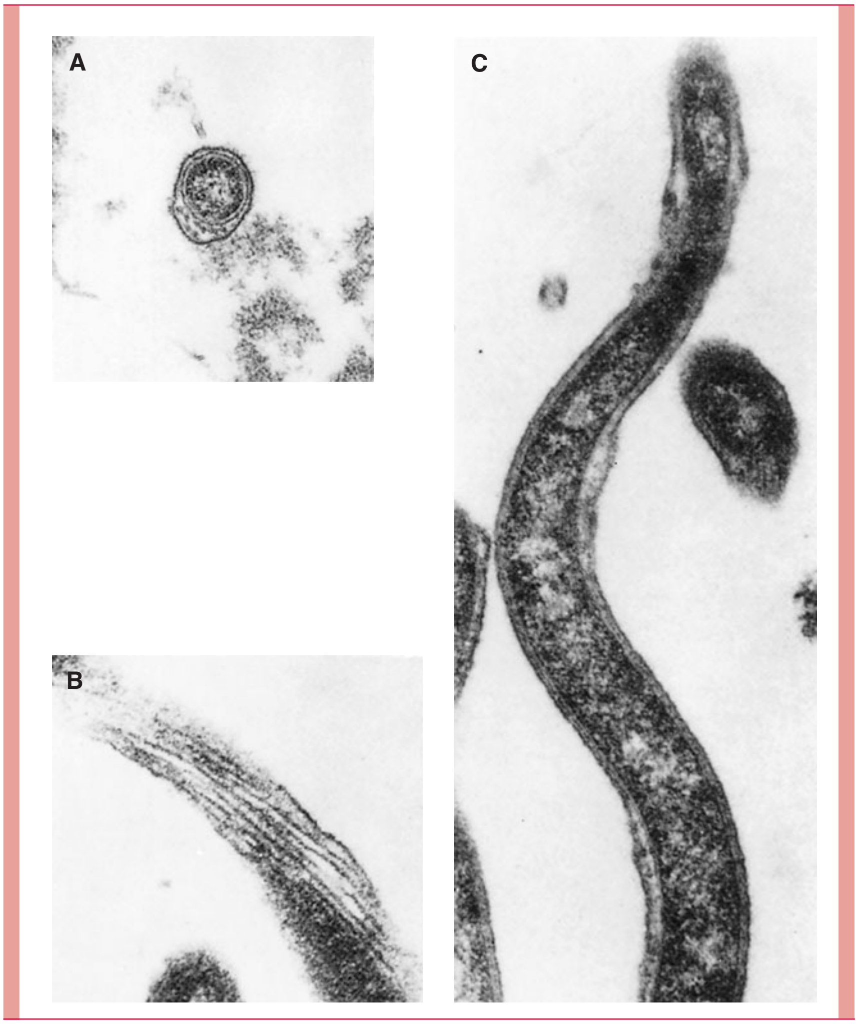


FIGURE 27–1
Spirochete of Lyme disease. Original magnification × 40,000. **A, B.** Note endoflagella. **C.** Note outer membrane. (*Reprinted with permission from Dr. Steere AC.* N Engl J Med *1983;308:736.*)

and the arrangement of flagella. Modern DNA homology and ribosomal RNA analyses have supported these groupings.

## SPIROCHETAL DISEASES

Some spirochetes are free living; some are members of the normal flora of humans and animals. The oral cavity, particularly the dental crevice, harbors a number of species of the genera *Treponema* and *Borrelia* as part of its normal flora. Under unusual conditions these spirochetes together with anaerobes in the normal flora can cause necrotizing, ulcerative infection of the gums, oral cavity, or pharynx (Vincent's infection, trench mouth). The pathogenesis of these opportunistic infections is not understood but they are correlated with immunocompromise, severe malnutrition, and neglect of basic hygiene. The term "trench mouth" refers to the occurrence of these infections in troops under the appalling conditions that existed in the trenches during World War I.

Many are part of oropharyngeal flora

Overgrowth causes trench mouth

The major spirochetal diseases are caused by selected species of three genera which are not found in the normal flora, *Treponema (T. pallidum), Leptospira (L. interrogans),* and *Borrelia (B. recurrentis, B. hermsii,* and *B. burgdorferi).* Most *Borrelia* and *Leptospira* infections are zoonoses transmitted from wild and domestic animals. *T. pallidum* is a strict human pathogen transmitted by sexual contact. Some rare nonvenereal treponemal diseases are summarized in Appendix 27–1.

Diseases are zoonoses or venereal

# Treponema pallidum

*T. pallidum* is the causative agent of syphilis, a venereal disease first recognized in the 16th century as the "great pox" that rapidly spread through Europe in association with urbanization and military campaigns. Some argue that it was brought back from the New World by the sailors with Christopher Columbus. Its extended course and the protean, often dramatic nature of its findings (genital ulcer, ataxia, dementia, ruptured aorta) are due to a state of balanced parasitism which spans decades. The cause of syphilis is actually a subspecies (*T. pallidum* subsp. *pallidum*) closely related to other agents which cause rare nonvenereal treponematoses which are summarized in Appendix 27–1. *T. pallidum* is used here to indicate the *pallidum* subspecies.

Syphilis represents an extended balance of parasitism and disease

## BACTERIOLOGY

### MORPHOLOGY

*T. pallidum* is a slim (0.15 $\mu$m) spirochete 5 to 15 $\mu$m long with regular spirals that resemble corkscrews with a wavelength (1 $\mu$m) and amplitude (0.3 $\mu$m). The organism is readily seen only by immunofluorescence, darkfield microscopy, or silver impregnation histologic techniques. Live *T. pallidum* cells show characteristic slow, rotating motility with sudden 90-degree angle flexion that suggests a gentleman quickly bowing at the waist.

Corkscrew spiral demonstrates characteristic motility and flexion

### GROWTH AND METABOLISM

*T. pallidum* has not been grown in the absence of cultured mammalian cells. Although it prefers low oxygen tensions, it is not a strict anaerobe. With careful control of oxygen tension and pH, the organism has now been shown to multiply through several generations in primary cell culture, but is difficult to subculture. Growth is slow, with a mean generation time of about 30 hours. Information about its metabolic properties is limited because of the extreme difficulty in obtaining sufficient organisms for study. [In vivo growth is usually achieved by injection into rabbit testes—a source of antigens for specific antibody testing.]

Growth is limited and slow in cell culture

*T. pallidum* is extremely susceptible to any deviation from physiologic conditions. It dies rapidly on drying and is readily killed by a wide range of detergents and disinfectants. The lethal effect of even modest elevations of temperature (41° to 42°C) was the basis of fever therapy early in the last century. These fragile properties account for its almost exclusive transmission by direct contact.

Heat, drying, and disinfectants kill quickly

### ANTIGENIC STRUCTURE

Many studies of *T. pallidum* suggest its surface is inert lacking proteins and other exposed antigens. The search for such structures has been hampered by the inability to grow the organism free of animals or cell culture. This not only makes potential antigens difficult to isolate and purify but also introduces the issues of whether a component has been derived from the host or the bacteria. The outer membrane of *T. pallidum* contains antigenic transmembrane proteins and lipoproteins but in quantities that are approximately 100-fold less than other Gram-negative bacteria such as *Escherichia coli.*

Outer membrane and surface are relatively devoid of proteins

## SYPHILIS

CLINICAL CAPSULE

Syphilis is typically acquired by the direct contact of mucous membranes during sexual intercourse. The disease begins with a lesion at the point of entry, usually a genital ulcer. After healing of the ulcer, the organisms spread systemically, and the disease returns weeks later as a generalized maculopapular rash called secondary syphilis. The disease then enters a second eclipse phase called latency. The latent infection may be cleared by the immune system or reappear as tertiary syphilis years to decades later. Tertiary syphilis is characterized by focal lesions whose locale determines the injury. Isolated foci in bone or liver may be unnoticed, but infection of the cardiovascular or nervous systems can be devastating. Progressive dementia or a ruptured aortic aneurysm are two of many fatal outcomes of untreated syphilis.

### EPIDEMIOLOGY

*T. pallidum* is an exclusively human pathogen under natural conditions. In most cases, infection is acquired from direct sexual contact with an individual who has an active primary or secondary syphilitic lesion. Partner notification studies suggest transmission occurs in over 50% of sexual contacts where a lesion is present. Less commonly, the disease may be spread by nongenital contact with a lesion (eg, of the lip), sharing of needles by intravenous drug users, or transplacental transmission to the fetus within approximately the first 3 years of the maternal infection. Late disease is not infectious. Modern screening procedures have essentially eliminated blood transfusion as a source of the disease. Since 1990, the number of reported new cases of syphilis in the United States has been declining; levels are now below 40,000 per year. Approximately 20% of cases are primary or secondary syphilis; the remainder are latent or tertiary disease. Worldwide, syphilis remains a major public health problem, with an estimated 12 million new cases annually.

Transmission is by contact with mucosal surfaces or blood

Congenital infection is transplacental

Tertiary lesions are not transmitted

### PATHOGENESIS

When certain strains of *T. pallidum* are inoculated into the skin, cornea, or testicle of animals lesions resembling primary syphilis can be produced, but there is no model for the other stages of disease. Because of our inability to grow the organism in culture, our knowledge of disease mechanisms is limited to the following extrapolations based on observations of human disease and experiments in animal models.

Experimental systems are limited

The spirochete reaches the subepithelial tissues through inapparent breaks in the skin or possibly by passage between the epithelial cells of mucous membranes, where it multiplies slowly with little initial tissue reaction. This may be due to the relative paucity of exposed antigens on the surface of the organism, but no specific reasons are known. As lesions develop, the basic pathologic finding is an endarteritis. The small arterioles show swelling and proliferation of their endothelial cells. This reduces or obstructs local blood supply, probably accounting for the necrotic ulceration of the primary lesion and subsequent destruction at other sites. Dense, granulomatous cuffs of lymphocytes, monocytes, and plasma cells surround the vessels. Although the primary lesion heals spontaneously the bacteria disseminate to other organs by way of local lymph nodes and the bloodstream.

Access is through mucosal breaks

Slow multiplication produces endarteritis, granulomas

Ulcer heals but spirochetes disseminate

For reasons that are not understood, syphilis is then silent until the disseminated secondary stage develops and then silent again with entry into latency. Although evasion of host defenses is clearly taking place, the mechanisms involved are unknown. The appearance of new epitopes in outer membrane proteins (OMPs) has been demonstrated during the course of experimental infections, but *T. pallidum* strains found in secondary lesions have not been demonstrated to differ antigenically from those in primary lesions. The organism has been observed to bind host proteins, immunoglobulins, and complement to its surface without sacrificing viability or motility. *T. pallidum* may be able to put on a host-like molecular "disguise" and thus avoid immune recognition.

Latent periods may be due to surface binding of host components

Injury is due to prolonged hypersensitivity responses

The inflammatory response to immune complexes, spirochetal lipoproteins, and complement in arteriolar walls accounts for some of the injury in syphilitic lesions. The granulomatous nature of the lesions in late syphilis is consistent with injury caused by delayed-type hypersensitivity responses prolonged by persistence of the spirochetes. In all of this, no toxins, virulence factors, or other molecules can yet be linked with specific features of syphilis.

## IMMUNITY

Immunity develops slowly and incompletely

Clinical observations suggest an immune response in syphilis which is vigorous but slow and imperfect. Immunity to reinfection does not appear until early latency, and for at least one third of those infected the subsequent host response is successful in clearing most but not all of the treponemes.

Antibodies to OMP are associated with reinfection resistance

Development of cell-mediated immunity clears lesions

Variable T lymphocyte suppression may link to stages

The immune mechanisms involved are far from clear but appear to involve both humoral and cell-mediated responses. Resistance to reinfection is correlated with appearance of antitreponemal antibody which is able to immobilize and kill the organism. Exposed treponemal OMPs are the most probable target of these antibodies. Cell-mediated responses appear to be dominant in syphilitic lesions with T lymphocytes (CD4+ and CD8+) and macrophages the primary cell types present. Activated macrophages play a major role in the clearance of *T. pallidum* from early syphilis lesions. The relapsing course of primary and secondary syphilis may reflect shifts in the balance between developing cellular immunity and suppression of T lymphocytes. Syphilis in immunocompromised patients such as those with acquired immunodeficiency syndrome may present with unusually aggressive or atypical manifestations.

# SYPHILIS: CLINICAL ASPECTS

## MANIFESTATIONS

### Primary Syphilis

Painless, indurated ulcer starts the disease

Heals spontaneously after weeks

The primary syphilitic lesion is a papule which evolves to an ulcer at the site of infection. This is usually the external genitalia or cervix but could be in the anal or oral area depending on the nature of sexual contact. The lesion becomes indurated and ulcerates but remains painless although slightly sensitive to touch. The fully developed ulcer with a firm base and raised margins is called the chancre (Fig 27–2). Firm, nonsuppurative, painless enlargement of the regional lymph nodes usually develops within 1 week of the primary lesion and may persist for months. The median incubation period from contact until appearance of the primary lesion is about 3 weeks (range 3 to 90 days). It heals spontaneously after 4 to 6 weeks.

FIGURE 27–2
Primary syphilis. A syphilitic chancre is shown on the glans penis. Note the sharp edge and raw base of the ulcer.

## Secondary Syphilis

Secondary or disseminated syphilis develops 2 to 8 weeks after the appearance of the chancre. The primary lesion has usually healed but may still be present. This most florid form of syphilis is characterized by a symmetric mucocutaneous maculopapular rash and generalized nontender lymph node enlargement with fever, malaise and other manifestations of systemic infection. Skin lesions are distributed on the trunk and extremities, often including the palms, soles, and face, and can mimic a variety of infectious and noninfectious skin eruptions. About one third of patients develop painless mucosal warty erosions called **condylomata lata.** These erosions usually develop in warm, moist sites such as the genitals and perineum. All the lesions of secondary syphilis are teeming with spirochetes and are highly infectious. They resolve spontaneously after a few days to many weeks, but the infection has resolved in only one third of patients. In the remaining two thirds of patients, the illness enters the latent state.

Lymphadenopathy and maculopapular rash are generalized

Spirochetes are abundant

Lesions resolve but disease continues in one third of patients

## Latent Syphilis

Latent syphilis is by definition a stage where there are no clinical manifestations but continuing infection is evidenced by serologic tests. In the first few years latency may be interrupted by progressively less severe relapses of secondary syphilis. In late latent syphilis (>4 years) relapses cease, and patients become resistant to reinfection. Transmission to others is possible from relapsing secondary lesions and by transfusion or other contact with blood products. Mothers may transmit *T. pallidum* to their fetus throughout latency. About one third of untreated cases do not progress beyond this stage.

Secondary relapses interrupt latency

Blood-borne transmission risk continues

## Tertiary Syphilis

Another one third of patients with untreated syphilis develop tertiary syphilis. The manifestations may appear as early as 5 years after infection but characteristically occur after 15 to 20 years. The manifestations depend on the body sites involved the most important of which are the nervous and cardiovascular systems.

**Neurosyphilis** is due to the damage produced by a mixture of meningovasculitis and degenerative parenchymal changes in virtually any part of the nervous system. The most common entity is a chronic meningitis with fever, headache, focal neurologic findings, and increased cells and protein in the cerebrospinal fluid (CSF). Cortical degeneration of the brain causes mental changes ranging from decreased memory to hallucinations or frank psychosis. In the spinal cord demyelination of the posterior columns, dorsal roots, and dorsal root ganglia produces a syndrome called tabes dorsalis which includes ataxia, wide-based gait, foot slap, and loss of the sensation. The most advanced central nervous system (CNS) findings include a combination of neurologic deficits and behavioral disturbances called **paresis,** which is also a mnemonic (**p**ersonality, **a**ffect, **r**eflexes, **e**yes, **se**nsorium, **i**ntellect, **s**peech) for the myriad of changes seen.

Chronic meningitis leads to degenerative changes, and psychosis

Demylination causes peripheral neuropathies

Syphilitic paresis has many signs

**Cardiovascular syphilis** is due to arteritis involving the vasa vasorum of the aorta causing a medial necrosis and loss of elastic fibers. The usual result is dilatation of the aorta and aortic valve ring. This in turn leads to aneurysms of the ascending and transverse segments of the aorta and/or aortic valve incompetence. The expanding aneurysm can produce pressure necrosis of adjacent structures or even rupture. A localized, granulomatous reaction to *T. pallidum* infection called a **gumma** may be found in skin, bones, joints, or other organ. Any clinical manifestations are related to the local destruction as with other mass-producing lesions, such as tumors.

Aortitis leads to aneurysm

Gummas are destructive, localized granulomas

## Congenital Syphilis

Fetuses are susceptible to syphilis only after the fourth month of gestation, and adequate treatment of infected mothers before that time prevents fetal damage. Because active syphilitic infection is devastating to infants, routine serologic testing is performed in early pregnancy and should be repeated in the last trimester in women at high risk of acquiring syphilis. Untreated maternal infection may result in fetal loss or congenital syphilis, which is analogous to secondary syphilis in the adult. Although there may be

Rhinitis, rash, and bone changes are common

Serologic screening and treatment is preventative

no physical finding at all, the most common are rhinitis and a maculopapular rash. Bone involvement produces characteristic changes in the architecture of the entire skeletal system (saddle nose, saber shins). Anemia, thrombocytopenia, and liver failure are terminal events.

## DIAGNOSIS

### Microscopy

Darkfield requires experience and fluid from deep in lesion

May be negative due to small numbers

*T. pallidum* can be seen by darkfield microscopy in primary and secondary lesions, but the execution of this procedure requires experience and attention to detail. The suspect lesion must be cleaned and abraded to produce a serous transudate from below the surface of the ulcer base. This material can be captured in a capillary tube or placed directly on a microscope slide if a darkfield setup is close at hand. The microscopist must observe the corkscrew morphology and characteristic motility to make a diagnosis (Fig 27–3). A negative examination does not exclude syphilis, because to be readily seen, the fluid must contain thousands of treponemes per milliliter. Darkfield microscopy of oral and anal lesions is not recommended because of the risk of misinterpretation of other spirochetes present in the normal flora. Direct fluorescent antibody methods have been developed but are available only in certain centers.

### Serologic Tests

Tests may or may not use treponemes

Most cases of syphilis are diagnosed serologically using serologic tests that detect antibodies directed at either lipid or specific treponemal antigens. The former are called nontreponemal tests, and the latter are referred to as treponemal tests. Their use in screening, diagnosis, and therapeutic evaluation of syphilis has been refined over many decades.

### Nontreponemal Tests

Reagin antibody reacts with cardiolipin, a lipid complex

Antibody level peaks in secondary syphilis

Nonspecfic reactions linked to autoimmune diseases

Nontreponemal tests measure antibody directed against **cardiolipin,** a lipid complex so called because one component was originally extracted from beef heart. Anticardiolipin antibody is called **reagin,** and the tests which detect it depend on immune flocculation of cardiolipin in the presence of other lipids. The most common nontreponemal tests are the rapid plasma reagin (RPR) and the Venereal Disease Research Laboratory (VDRL). They become positive in the early stages of the primary lesion and, with the possible exception of some patients with advanced human immunodeficiency virus (HIV) infection, are uniformly positive during the secondary stage. They slowly wane in the later stages of the disease. In neurosyphilis, VDRL tests on CSF may be positive when the serum VDRL has reverted to negative. Nontreponemal tests are nonspecific; they may become positive in a variety of autoimmune diseases or in diseases involving substantial tissue or liver destruction, such as lupus erythematosus, viral hepatitis, infectious mononucleosis, and malaria. False-positive results can also occur occasionally in pregnancy and in patients with HIV infection.

Titer is used to follow therapy

Sensitivity and low cost make nontreponemal tests preferred for screening, but if positive, they must be confirmed by one of the more specific treponemal tests described below. They are also valuable for following treatment because the height of the antibody titer is directly related to activity of disease. With successful antibiotic therapy nontreponemal serologies slowly revert to negative.

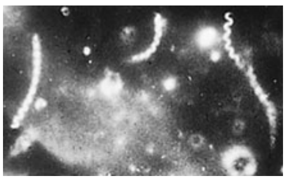

FIGURE 27–3
*Treponema pallidum* seen by darkfield microscopy. The darkfield method creates a bright halo around the corkscrew-shaped spirochetes.

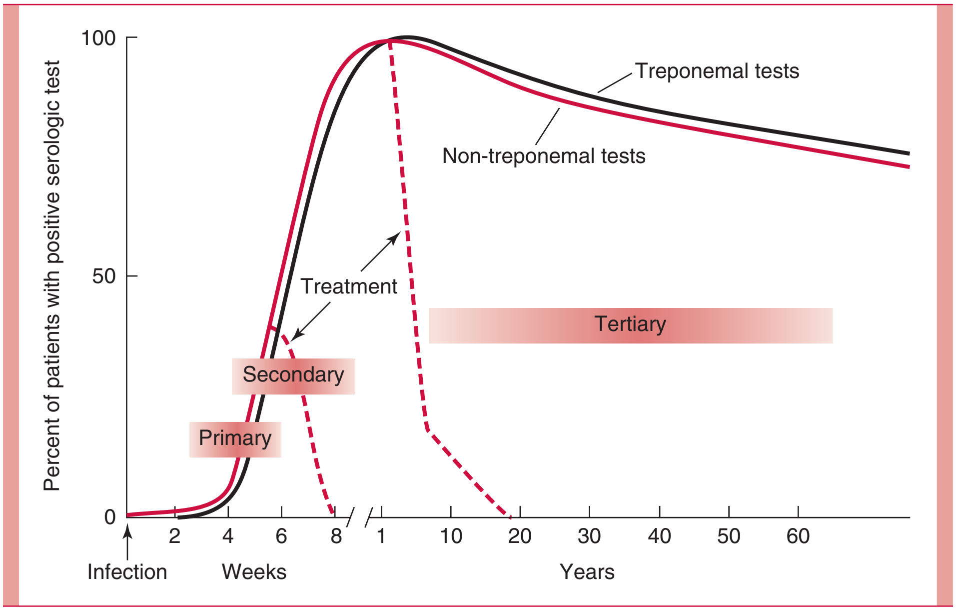


FIGURE 27–4
Treponemal and nontreponemal tests in syphilis. The time course of treated and untreated symphilis in relation to serologic tests is shown. The non-treponemal tests (VDRL, RPR) rise during primary syphilis and reach their peak in secondary syphilis. They slowly decline with advancing age. With treatment they revert to normal over a few weeks. The treponemal tests (FTA-ABS, MHA-TP) follow the same course but remain elevated even following successful treatment.

## Treponemal Tests

Treponemal tests detect antibody specific to *T. pallidum* such as an indirect immunofluorescent procedure called the fluorescent treponemal antibody (FTA-ABS) which uses spirochetes fixed to slides. The "ABS" refers to an absorption step that removes nonspecific antispirochetal antibodies often found in normal serum. Another method, the microhemagglutination test for *T. pallidum* (MHA-TP), uses antigens attached to the surface of erythrocytes, which then agglutinate in the presence of specific antibody.

*T. pallidum* is used as the antigen

Treponemal tests are considerably more specific than the cardiolipin-based nontreponemal tests. Their primary role in diagnosis is to confirm positive RPR and VDRL results obtained in the evaluation of a patient suspect for syphilis or in screening programs. They are not useful for screening or following therapy because, once positive, they usually remain so for life. Thus, if nontreponemal tests can be thought of as the measure of active syphilis, treponemal tests are the indelible print of sin. The time course of serologic tests in the various stages of syphilis is illustrated in Figure 27–4. Until recently, it was believed that a negative treponemal test excluded the possibility of prior syphilis.

Positive result confirms RPR and VDRL

Remain positive for life

The use of serologic tests in the diagnosis of congenital syphilis is complicated by the presence of IgG antibodies in infants, who acquire it transplacentally from their mothers. If available, treponemal IgM tests are useful in establishing the presence of an acute infection in infants.

IgM is used to diagnose congenital syphilis

## TREATMENT AND PREVENTION

*T. pallidum* remains exquisitely sensitive to penicillin, which is the preferred treatment in all stages. In primary, secondary, or latent syphilis persons hypersensitive to penicillin may be treated with tetracyclines, erythromycin, or cephalosporins. The efficacy of agents other than penicillin has not been established in tertiary or congenital syphilis. It is recommended that penicillin-hypersensitive patients with neurosyphilis or congenital syphilis be desensitized rather than use an alternate antimicrobial. Safe sex practices are as effective for prevention of syphilis as they are for other sexually transmitted diseases. Their use to prevent HIV infection probably accounts for much of the recent decline in new syphilis cases. The development of a vaccine awaits greater understanding of pathogenesis and immunity.

Penicillin is preferred

Safe sex blocks transmission

## *LEPTOSPIRA INTERROGANS*

### BACTERIOLOGY

Loose spirals seen in darkfield

*L. interrogans* is the member of the genus *Leptospira* that is pathogenic to humans and animals. There are other free-living species of *Leptospira*. This species is a slim (approximately 0.15 $\mu$m) spirochete 5 to 15 $\mu$m long, with a single axial filament; fine, closely wound spirals; and hooked ends. It is not visualized with the usual staining procedures, and detection is best accomplished using darkfield microscopy. It can be grown in aerobic culture using certain special enriched semisolid media.

Multiple serogroups have geographic associations

Survives in water

*L. interrogans* has multiple serogroups and over 200 serotypes, many of which were previously accorded species status (eg, *L. icterohaemorrhagiae, L. canicola, L. pomona*) based on geographic occurrence, differences in host species, and associated clinical syndromes. The distinction between serogroups and serotypes is of epidemiologic and epizoologic importance but has no clinical significance. *Leptospira interrogans* can survive days or weeks in some waters in the environment at a pH above 7.0. Acidic conditions, such as those that may be found in urine, rapidly kill the organism. It is highly sensitive to drying and to a wide range of disinfectants.

### LEPTOSPIROSIS

CLINICAL CAPSULE

Leptospirosis is a systemic flu-like illness associated with water contaminated by animal urine. It begins with fever, nausea, vomiting, headache, abdominal pain, and severe myalgia. In severe cases, a second phase is characterized by impaired hepatic and renal function with jaundice, prostration, and circulatory collapse. The CNS is often involved, with stiff neck and inflammatory changes in the cerebrospinal fluid.

#### EPIDEMIOLOGY

Animals are reservoir

Water is transmission route

Leptospirosis is a worldwide disease of a variety of wild and domestic animals particularly rodents, cattle, and dogs. It is usually transmitted to humans through water contaminated with animal urine. Secondary human-to-human transmission occurs rarely. Individuals who are exposed to animals (eg, farmers, veterinarians, slaughterhouse employees) are at increased risk, although most clinical cases in North America are now associated with recreational exposure to contaminated water (eg, irrigation ditches or other bodies of water receiving farmland drainage). In tropical areas leptospirosis may account for up to 10% of hospital admission, particularly following rains or floods.

#### PATHOGENESIS AND IMMUNITY

Enters through small mucosal breaks

Blood and CNS spread is common

The organism gains entrance to the tissues through small skin breaks, the conjunctiva or, most commonly, through ingestion and the upper alimentary tract mucosa. The active motility of the hooked ends driven by periplasmic flagella may allow the organism to burrow into tissues. The organisms spread widely through the bloodstream to all parts of the body including the CSF. In animals they colonize the proximal renal tubule, from which they are shed into the urine, facilitating transmission to new hosts. The kidney is also a target organ in human disease causing tubular infection and interstitial nephritis.

Antibody may be part of disease

Clearing of the bacteremia is associated with the appearance of circulating antibody but little else is known of immune mechanisms. Antibody is also rising during the second phase of the disease which suggests an immunologic component to its pathogenesis. This is supported by absence of response to antimicrobics when given at this stage and failure usually to recover the organism from the CSF in cases of leptospiral meningitis.

## LEPTOSPIROSIS: CLINICAL ASPECTS

### MANIFESTATIONS

Most infections are subclinical and detectable only serologically. After an incubation period of 7 to 13 days, an influenza-like febrile illness with fever, chills, headache, conjunctival suffusion, and muscle pain develops in persons who become ill. This disease is associated with bacteremia. Leptospires are also found in the CSF at this stage, but without clinical or cytologic evidence of meningitis. The fever often subsides after about a week coincident with the disappearance of the organisms from the blood but may recur with a variety of clinical manifestations depending partly on the serogroup involved. This second phase of the disease usually lasts 3 or more weeks and may present as an aseptic meningitis resembling viral meningitis (see Chapter 67) or as a more generalized illness with muscle aches, headache, rash, pretibial erythematous lesions, biochemical evidence of hepatic and renal involvement, or all of these. In its most severe form (Weil's disease), there is extensive vasculitis, jaundice, renal damage, and sometimes a hemorrhagic rash. The mortality in such cases may be as high as 10%.

Initial disease is flu-like

Meningitis and muscle aches last for weeks

Hemorrhagic rash is linked to fatal outcome

### DIAGNOSIS

The diagnosis of leptospirosis is primarily serologic. Although the spirochetes could theoretically be detected, darkfield examination of body fluids is not recommended. The yield is very low and the chance for confusion with fibrin and debris is significant. Likewise, leptospires can be isolated from the blood, CSF, or urine, but culture is rarely attempted because the organisms take weeks to grow in a special medium which few laboratories bother to stock. The standard serologic test (microscopic agglutination) is also limited to reference laboratories. A simpler slide agglutination is less specific but may be suggestive of infection in the presence of a compatible clinical picture.

Serologic tests are limited to reference laboratories

### TREATMENT

Penicillin, ampicillin, and erythromycin are effective for severe forms of leptospirosis. Tetracyclines (including doxycycline) are also recommended for milder disease. Third-generation cephalosporins and other antimicrobics are active in vitro but are not yet backed up by sufficient clinical experience.

Multiple antimicrobics are effective

### PREVENTION

Vaccines are used in cattle and household pets to prevent the disease, and this has reduced its occurrence in humans. Doxycycline, given once weekly, prevents leptospirosis in individuals working in high-risk environments for short periods. Other measures include rodent control, drainage of waters known to be contaminated, and care on the part of those subject to occupational exposure to avoid ingestion or contamination with *L. interrogans.*

Rodent and water control are important

## BORRELIA

More than 15 species of *Borrelia* have been associated with human disease, and other species are responsible for similar diseases in animals. *B. burgdorferi* is the cause of Lyme disease. Other members of the genus cause relapsing fever, an illness with intermittent fevers and

Relapsing fever and Lyme disease caused by different species

little else. The relapsing fevers differ in their specific vector and geographic distribution. The human body louse is the vector for *B. recurrentis,* but the remainder of the relapsing fevers are linked to several ticks and species of *Borrelia;* these are discussed together here as *B. hermsii,* the most common cause of relapsing fever in North America.

## BACTERIOLOGY

### MORPHOLOGY, STAINING, AND CULTURE

Loose, irregular spirals take common stains

*Borrelia* are long (10 to 30 $\mu$m), slender (0.2 to 0.5 $\mu$m) spirochetes containing multiple (7 to 20) axial flagella. In contrast to *Treponema* and *Leptospira,* its spirals form loose, irregular waves. The basic organizational structure of the cell and its motility conform to that of the other Gram negative spirochetes, but unlike the others, *Borrelia* are readily demonstrated by common staining methods like the Giemsa or Wright stains. *Borrelia* are microaerophilic and have been successfully grown in liquid or semisolid media containing N-acetylglucosamine and long-chain fatty acids.

### ANTIGENIC STRUCTURE AND VARIATION

Surface proteins undergo antigenic variation

Recombination between linear plasmids leads to altered protein

The outer membrane of all *Borrelia* species contains abundant outer membrane proteins and lipoproteins. In some species these surface proteins have been observed to vary antigenically at a frequency too high to be explained by simple mutation. Experiments with *B. hermsii* have demonstrated up to 40 antigenically distinct variants of the same protein arising from a single cell. The genetic mechanism for this antigenic variation involves recombination between the distinctive linear plasmids found in many *Borrelia* species. Multiple copies of the genes for these proteins are present. Some genes express the protein while others are "silent" because they lack crucial promoter sequences. When structural sequences from a silent gene are transferred by recombination to an expressing gene on another plasmid, the protein expressed is altered in a which may make it antigenically different. This mechanism resembles that described for trypanosomes (see Chapter 54) and gonococcal pili (see Chapter 20).

## RELAPSING FEVER

CLINICAL CAPSULE

Relapsing fever is an illness with fever, headache, muscle pain, and weakness but no signs pointing to any organ system. It lasts about 1 week and returns a few days later. The relapses may continue for as many as four cycles. During each relapse, spirochetes are present in the bloodstream. The causative *Borrelia* species are transmitted to humans from ticks or body lice.

### EPIDEMIOLOGY

Body lice or ticks transmit the spirochete

Relapsing fever occurs in two forms linked to the mode of transmission and the *Borrelia* species involved. The louse-borne form usually appears in epidemics, because of circumstances connected with body lice, whereas the tick-borne form does not. For this reason, the two forms are sometimes called epidemic (louse-borne) and endemic (tick-borne) relapsing fever. Here they will be identified simply by the insect involved.

Tick reservoir feeds on rodents and small animals

The occurrence and distribution of tick-borne relapsing fever are determined by the biology of the relevant tick species and its relationship to the primary *Borrelia* reservoir in rodents and other small animals (rabbits, birds, lizards). Ticks may remain infectious for several years even without feeding and transovarial passage to their progeny extends the infectious chain even further. Humans are infected when they accidentally enter this cycle and are bitten by an infected tick. The bite is painless and the feeding period is brief (<20 minutes). Because the ticks usually feed at night, cases are most often associated with overnight recreational forays into wild, wooded areas. The largest outbreak in the United States involved National Park employees and tourists who slept in tick- and rodent-infested cabins on the Northern Rim of the Grand Canyon.

Nighttime painless tick bite transmits bacteria

The epidemiologic conditions associated with louse-borne relapsing fever are much more exacting. The human body louse has no other host, infected lice live no more than 2 months, and there is no transovarial passage to progeny. *B. recurrentis* is the only species involved. Lice are infected from human blood but the spirochetes multiply in their hemolymph, not any of the feeding parts or excrement. This means they can infect another human only if the louse is crushed by scratching and the *Borrelia* reach a superficial wound or mucosal surface. Infected lice must be passed human to human for the disease to persist. These conditions are met by circumstances that combine overcrowding with extremely low levels of general hygiene. War, other kinds of social breakdown, and dire poverty are the prime associates. Currently, this variety of relapsing fever appears to be limited to east and central Africa and the Peruvian Andes.

Body lice infected from human blood

Lice must be transferred from human to human

## PATHOGENESIS

The disease manifestations develop at times when thousands of spirochetes are circulating per milliliter of blood. The febrile illness has endotoxin-like features, but the exact mechanisms of disease are unknown. Between episodes the organisms disappear from the blood and are sequestered in internal organs only to reappear during relapses. The OMPs are antigenically different with each relapse. The relapsing cycles correlate with antibody production to the new protein followed by clearing followed by emergence of a new antigenic type.

Spirochetes appear in blood

Altered OMPs occur with relapse

## IMMUNITY

Immunity to the disease is largely humoral and appears to involve lysis of the organism in the presence of complement. The disease is controlled when variants from the antigenic repertoire are no longer able to escape the immune response.

Antibody eventually controls disease

# RELAPSING FEVER: CLINICAL ASPECTS

## MANIFESTATIONS

After a mean incubation period of 7 days, massive spirochetemia develops, with high fever, rigors, severe headache, muscle pains, and weakness. The febrile period lasts about 1 week and terminates abruptly with the development of an adequate immune response. The disease relapses 2 to 4 days later, usually with less severity, but following the same general course. Tick-borne relapsing fever is usually limited to one or two relapses, but with louse-borne disease three or four may occur.

Fever, headache and muscle pain last 2–4 days

Louse-borne relapsing fever is more severe than tick-borne disease, possibly because of predisposing social conditions. Fatalities are rare in tick-borne disease but may be as high as 40% in untreated louse-borne fever. Fatal outcomes are due to myocarditis, cerebral hemorrhage, and hepatic failure.

Louse-borne is more severe

## DIAGNOSIS

Diagnosis is readily made during the febrile period by Giemsa or Wright staining of blood smears. The appearance of the spirochete among the red cells is characteristic. Cultural and animal inoculation procedures are also used for recovery of the infecting organism. Serodiagnostic tests are unhelpful.

Blood smears demonstrate *Borrelia*

## TREATMENT

Tetracycline and erythromycin are favored

The disease responds well to tetracycline or erythromycin therapy, and single-dose treatment with these agents can be effective. Jarisch–Herxheimer reactions are particularly common in the treatment of relapsing fever perhaps because of the height of the spirochetemia at the time of antibiotic administration.

## PREVENTION

Attention to ticks and general hygiene are important

Prevention of tick-borne relapsing fever involves attention to deticking, insecticide treatment, and rodent control around habitations, such as mountain cabins, shown to be associated with infection. Control of louse-borne relapsing fever involves delousing, particularly dusting of clothing with appropriate insecticides. Ultimately, improved hygiene stops outbreaks and prevents further occurrences.

# *BORRELIA BURGDORFERI*

## BACTERIOLOGY

Grows in microaerophilic atmosphere

Osps differ at stages of infection

*B. burgdorferi* is microaerophilic and can be grown with some difficulty in a specialized artificial culture medium. The doubling time under these conditions is long (8 to 24 hours), so growth for isolation takes many days to weeks. *B. burgdorferi* consists of at least 10 different subspecies (eg, *B. burgdorferi* sensu stricto, *B. afzelii, B.garini*), which differ in geographic distribution and some clinical manifestations. All will be referred to as *B. burgdorferi* here. As with other species of *Borrelia,* there are multiple classes of OMPs, many of which undergo antigenic variation. Recent studies have focused on a class called outer surface proteins (Osps), which have been linked to aspects of pathogenesis and immunity. Two of these, OspA and OspC, are differentially expressed depending on the stage of tick or mammalian infection.

## LYME DISEASE

CLINICAL CAPSULE

Acute Lyme disease is characterized by fever, a migratory "bull's eye" skin rash, muscle and joint pains, often with evidence of meningeal irritation. In a chronic form evolving over several years meningoencephalitis, myocarditis, and a disabling recurrent arthritis may develop. *B. burgdorferi* is transmitted to humans by *Ixodes* ticks.

## EPIDEMIOLOGY

Spirochetes are transmitted in tick–mouse–deer cycle

Ticks must feed on humans in the woods

*B. burgdorferi* exists in a complex cycle involving ticks, mice, and deer. Lyme disease occurs when the ticks feed on humans who enter their wooded habitat. The disease is endemic in several regions of the United States, Canada, and temperate Europe and Asia. Approximately 90% of the 10,000 to 15,000 cases reported each year in the United States occur in areas along the northeastern and mid-Atlantic seaboard, including Old Lyme, Connecticut, where the disease was first recognized. The majority of cases probably go unreported, particularly outside the primary endemic regions.

The primary reservoir of *B. burgdorferi* is rodents, particularly white-footed mice. Infection is transmitted by *Ixodes* ticks, whose complete life cycle involves rodents for the early stages and deer for adult maturation. In the spring, fertile female ticks, engorged from their blood meals, fall from their deer hosts to the ground and deposit their eggs. During the summer, the tick larvae seek out and obtain a blood meal from mice and the *B. burgdorferi* ingested by the larvae are maintained through the subsequent development stages of the tick. The following spring or summer, the small (1 to 2 mm) nymphs feed again on vertebrate hosts to obtain the blood required for maturation to adulthood. The engorged, satiated nymphs fall off their hosts and mature into adults by parasitizing available deer, thus completing a life cycle that has occupied a full 2 years. Vertebrates other than deer can be infected by both the adult and nymph stages of the tick, but human Lyme disease is acquired primarily from nymphs, because they are active at the time of year when humans are most likely to invade their ecosystem. Deer are essential to the mating and survival of the tick and thus the disease does not occur in areas in which deer are not abundant.

Ticks feed on mice and then deer

Adult and nymph stages can infect humans

No deer, no disease

## PATHOGENESIS

Because Lyme disease is a recently discovered disease with a complex biology, it is not surprising that the pathogenic mechanisms in humans remain to be established clearly. Studies in ticks have shown changes in the antigenic makeup of *B. burgdorferi* as it migrates from the midgut and salivary glands and again after it reaches mammalian tissue. OspA is the major outer surface protein expressed when *B. burgdorferi* resides in ticks, but its expression diminishes during tick engorgement, while OspC increases, so that by the time of transmission to hosts, OspC predominates. Although OspC has been shown to stimulate protective antibody in animals, its role in disease is unknown.

OspA predominates in ticks

Shift to OspC is completed at vertebrate transmission

Some candidate adhesins of *B. burgdorferi* could be important in the early stages of human infection. These surface proteins and lipoproteins have been shown to mediate attachment to integrins, platelets, and collagen-associated elements of the extracellular matrix (ECM). Other molecules which bind plasmin to the spirochete surface may activate host proteolytic systems and facilitate spread through the ECM to adjacent tissues. It is known that the outer membrane of the spirochete contains proteins and a toxic lipopolysaccharide that differs from the usual Gram-negative endotoxin. The spirochetal peptidoglycan has inflammatory properties, survives considerable periods in tissues, and may contribute to arthritis when deposited in joint tissues.

Surface proteins bind to ECM

Lipopolysaccharide differs from other endotoxins

Peptidoglycan causes inflammation

Clinical investigations in patients with Lyme disease have noted modulation of immune responses, including inhibition of mononuclear and natural killer cell function, lymphocyte proliferation, and cytokine production. The ability of *B. burgdorferi* to downregulate deleterious immune responses could serve as a survival strategy or play a role in chronic disease. Chronic disease, particularly Lyme arthritis, has aspects of autoimmunity. One candidate cross-reactive autoantigen is OspA. The sera of individuals with Lyme arthritis but not other forms of arthritis react with epitopes present in OspA and with homologous epitopes in human leukocyte antigens (HLAs). A genetic basis for this linkage is suggested by the statistical association between chronic arthritis and certain HLA types. Such theories must be reconciled with the downregulation of OspA in mammalian infection and clarification of the role of many other candidate virulence factors.

Downregulation of immune function contributes to chronicity

Anti-OspA antibody has autoimmune activities

## IMMUNITY

The immune response to *B. burgdorferi* infection develops slowly with IgM followed by IgG antibody over weeks to months. Although immune-mediated killing by the classical complement pathway has been demonstrated the molecular target is unknown. Host neutrophils and macrophages can phagocytose opsonized spirochetes and induce a metabolic burst leading to spirochetal death. OspC elicits protective immunity in rodents, but this protection is short lived and ineffective against challenge with heterologous *B. burgdorferi* isolates. Antigens capable of eliciting broadly protective immune responses have not been identified.

Target of protective antibody is unclear

# LYME DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

Spreading lesion from bite site is most characteristic finding

Lyme borreliosis is a highly variable disease involving multiple body systems. It occurs in overlapping patterns that come and go at different times. The skin lesion spreading from the site of the tick bite is its most distinctive feature. Relapsing arthritis is the most persistent finding and the one most likely to become chronic. Lyme disease is rarely fatal, but if untreated, it is often a source of chronic ill health.

Erythema migrans and febrile aches mark acute disease

The primary lesion begins sometime in the first month after a tick bite, which is often unnoticed. A macule or papule appears at the site of the bite and expands to become an annular lesion with a raised, red border and central clearing forming a "bull's eye" pattern. As the bull's eye ring expands, the lesion known as **erythema migrans** forms. Along with the skin lesions fever, fatigue, myalgia, headache, joint pains, and mild neck stiffness are often present. Approximately 50% of untreated patients develop secondary skin lesions that closely resemble the primary one but are not at the site of the tick bite. In untreated patients, the skin lesions usually disappear over a period of weeks, but constitutional symptoms may persist for months.

Nerve palsies and cardiac findings appear later

Days, weeks, even months after the onset of the primary lesion, a second stage may develop in which involvement of the nervous or cardiovascular system may be superimposed. Neurologic abnormalities include a fluctuating meningitis, cranial nerve palsies, and peripheral neuropathy. Cardiac disease is usually limited to conduction abnormalities (atrioventricular block), but in some cases acute myocarditis can lead to cardiac enlargement. Both neurologic and cardiac abnormalities fluctuate in intensity but generally resolve completely in a matter of weeks.

Fluctuating arthritis may become chronic

Weeks to years after the onset of infection, arthritis marks the continuing state of the disease. It develops in almost two thirds of untreated patients. Typically, it too follows a fluctuating or intermittent course, generally involving the large joints, particularly the knees. The arthritis may become chronic with erosion of the bone and cartilage although the spirochetes are rarely demonstrable in the lesions. Less frequent chronic neurologic dysfunctions include subtle encephalitis affecting memory, mood, or sleep, and peripheral neuropathies.

## DIAGNOSIS

Culture and PCR are not yet practical

Presently, the diagnosis of early Lyme disease is based on exposure and typical clinical findings. Although *B. burgdorferi* can be cultured from erythema migrans skin lesions, blood, joint fluid, and CSF, few laboratories stock the special medium required. The spirochetes are seldom detected on any kind of direct microscopic examination. Polymerase chain reaction (PCR) procedures able to detect *B. burgdorferi*–specific DNA sequences in body fluids have been developed but are expensive and not standardized for routine use.

Serologic tests are not diagnostic

With culture generally unavailable, the diagnosis in later stages of disease usually rests on the demonstration of circulating antibodies to *B. burgdorferi*. Despite considerable progress these tests still lack the sensitivity and specificity to be considered more than supportive of a clinical diagnosis. The current recommendation is to first perform a sensitive screening test (enzyme immunoassay or fluorescent antibody) followed by a more specific Western blot. Even with this two-step approach, patients in the early stages may be seronegative and cross-reactive antigens may cause false-positive results.

## TREATMENT

Doxycycline and amoxicillin are the first-line antimicrobics for the treatment of early Lyme disease and arthritis. For individuals who cannot tolerate either of these agents, cefuroxime is a much more expensive alternative for oral therapy. Intravenous therapy with ceftriaxone or penicillin G is recommended for patients with neurologic involvement or cardiovascular findings such as atrioventricular heart block. The response to treatment is typically slow requiring the continuation of antimicrobics for 30 to 60 days.

Doxycycline and $\beta$-lactams are recommended

## PREVENTION

The most useful preventive measures in endemic areas are the use of clothes that reduce the likelihood of the infected nymph reaching the legs or arms, careful search for nymphs after potential exposure, and removal of the tick by its head with tweezers. Duration of tick attachment to humans is also a factor in transmission; the risk is greatest when the tick has been feeding for at least 48 to 72 hours. Some insect repellents may provide added protection. The risk of Lyme disease following a random tick bite is too low to justify administration of antimicrobics prophylactically.

Preventing bites and removing ticks are important

A vaccine for Lyme disease composed of recombinant OspA is now licensed for use in the United States. Unlike typical vaccines directed at a molecule known to be important in human infection, the Lyme disease vaccine is designed to act in the feeding tick, not the human. As indicated above OspA is expressed by *B. burgdorferi* in tick infection but downregulated when the spirochetes enter mammalian tissues. The antibodies stimulated by OspA immunization are intended to reach the midgut of feeding ticks and mediate killing of spirochetes before transmission can occur. In addition, the spirochetes may retain enough OspA to render them susceptible to the antibody shortly after transmission. The vaccine has been shown to be protective with an efficacy of approximately 75%. Its widespread use is controversial, because the benefits depend on the relation between the morbidity and cost of complicated cases and the incidence of Lyme disease in any population. By some estimates, the incidence in even the highest risk areas in Connecticut and Massachusetts does not justify immunizing everyone.

Vaccine is directed against the feeding tick

Cost-effectiveness of immunization is unclear

## ADDITIONAL READING

Centers for Disease Control and Prevention. Recommendations for the use of Lyme disease vaccine. *Morb Mortal Wkly Rep* 1999;48(RR-7):1–25. This supplement also includes a nice guide to the diagnosis of Lyme disease and a discussion of the cost-effectiveness from both the societal and payor perspective.

Horton JM, Blaser MJ. The spectrum of relapsing fever in the Rocky Mountains. *Arch Intern Med* 1985;145:871–875. This article analyzes the clinical manifestations, epidemiology, and treatment of 22 cases of tick-borne relapsing fever that occurred between 1944 and 1983 and describes in detail several of the later cases.

Levett PN. Leptospirosis. *Clin Microbiol Rev* 2001;14:296–326. This comprehensive review includes a good discussion of clinical and diagnostic features, with a nice figure that ties them together. The reader should not be dissuaded by the page count; almost half of the pages are for the reference list.

Shapiro ED, Gerber MA. Lyme disease. *Clin Infect Dis* 2000;31:533–542. This review emphasizes clinical and diagnostic features, including the complexities of interpreting serologic tests in immunized persons. There is also a short section on “Lyme anxiety.”

Singh AE, Romanowski B. Syphilis: Review with emphasis on clinical, epidemiologic, and some biologic features. *Clin Microbiol Rev* 1999;12:187–209. This comprehensive review gives a detailed account of clinical findings, diagnosis, and treatment for all stages of syphilis without shortchanging pathogenesis.

Steere AC. Lyme disease. *N Engl J Med* 2001;345:115–123. An excellent review of all aspects of the disease, including clinical features, biology, and prevention. The same issue of this journal also presents a study of antibiotic prophylaxis (pages 79–84) and a well-reasoned editorial on prevention (pages 133–134).

APPENDIX 27–1

## Nonvenereal Treponemes

| Disease | Cause | Major Geographic Location | Primary Lesion | Secondary Lesions | Tertiary Lesions |
|---|---|---|---|---|---|
| Bejel | *T. pallidum,* subspecies *endemicum*[a] | Middle East; arid, hot areas | Oral cavity[b] | Oral mucosa | Rare; gummatous lessions of skin, periosteum, bone, and joint |
| Yaws | *T. pallidum,* subspecies *pertenue* | Humid, tropical belt | Skin, papillomatous | Systemic; resemble syphilis | Rare; gummatous lesions of skin, periosteum, bone, and joint[c] |
| Pinta | *T. carateum* | Central and South America | Skin, erythematous papule | Skin; merge into primary lesion; altered pigmentation | Areas of altered skin pigmentation and hyperkeratoses |

[a] Probably a variant of that causing venereal syphilis.
[b] Often inapparent.
[c] Neurologic manifestations usually absent.

CHAPTER 28

# Mycobacteria

JAMES J. PLORDE

> The Captain of all these men of death that came against him to take him away, was the consumption; for it was that that brought him down to the grave.
>
> JOHN BUNYAN
> *The Life and Death of Mr. Badman*

*Mycobacterium* is a genus of Gram-positive bacilli that demonstrate the staining characteristic of acid-fastness. Its most important species, *Mycobacterium tuberculosis,* is the etiologic agent of tuberculosis, the "consumption" referred to above. One of the oldest and most devastating of human afflictions, tuberculosis remains a leading cause of infectious disease deaths worldwide today. A second mycobacterium, *Mycobacterium leprae,* is the causative agent of leprosy. A large number of less pathogenic species collectively referred to as "atypical mycobacteria" or "nontuberculous mycobacteria," are assuming increasing importance as disease agents in immunocompromised patients, particularly those with acquired immunodeficiency syndrome (AIDS).

## *MYCOBACTERIUM:* GENERAL CHARACTERISTICS

### BACTERIOLOGY

#### MORPHOLOGY AND STRUCTURE

The mycobacteria are slim, Gram-positive bacilli ($0.2-0.4 \times 2-10\ \mu m$). They are nonmotile, obligate aerobes that do not form spores. The cell wall contains peptidoglycan similar to that of other Gram-positive organisms, except that it contains *N*-glycolylmuramic, rather than *N*-acetylmuramic, acid. Attached to peptidoglycan are a myriad of branched chain polysaccharides, proteins, and lipids. Of particular importance are long-chain fatty acids called mycolic acids. The **mycolic acids,** for which the *myco*bacteria are named, make up more than 60% of the total cell wall mass and are distinctive for each species. Other lipid components include mycosides, sulfolipids, and **lipoarabinomannan (LAM),** a complex molecule extending from the plasma membrane to the surface. LAM is structurally and functionally analogous to the lipopolysaccharide of Gram-negative bacteria. Porin and other proteins are found throughout the cell wall.

Cell wall has high lipid content

Mycolic acids and LAM are characteristic

The cell wall lipids make the cell surface hydrophobic, rendering mycobacteria resistant to staining with basic aniline dyes unless they are applied with heat or detergents, or for prolonged periods of time. Once stained, however, mycobacteria resist decolorization with a mixture of 3% hydrochloric acid and 95% ethanol. These properties are described as **acid fastness** or, more properly, acid–alcohol fastness, and the bacteria possessing them are called acid-fast bacilli. Details are described in Chapter 15. This characteristic allows mycobacteria to be readily distinguished from other genera by microscopic examination of smears stained with carbol fuchsin (Ziehl–Neelsen/Kinyoun techniques), or with the more recently introduced fluorochromes (auramine–rhodamine). Organisms stained with the latter reagents fluoresce brightly when viewed through an appropriate microscope, making the organisms more visually apparent and, thus, decreasing the time required for their detection.

Difficult to stain, but once stained, difficult to decolorize

Acid fastness distinguishes from most other bacteria

## GROWTH

The most important pathogen, *M. tuberculosis,* shows enhanced growth in 10% carbon dioxide and at a pH of about 6.5 to 6.8. Nutritional requirements vary among species and range from the ability of some nonpathogens to multiply on the washers of water faucets to the strict intracellular parasitism of *M. leprae,* which does not grow in artificial media or cell culture. Mycobacteria grow more slowly than most human pathogenic bacteria because of their hydrophobic cell surface, which causes them to clump and inhibits permeability of nutrients into the cell. Addition of a surfactant (Tween 80) to cultures of *M. tuberculosis* wets the surface and leads to dispersed and more rapid growth.

Strict aerobes

Many species grow slowly

## CLASSIFICATION

Until recently, mycobacterial classification has been based on a constellation of phenotypic characteristics, including nutritional and temperature requirements, growth rates, pigmentation of colonies grown in light or darkness, key biochemical tests, the cellular constellation of free fatty acids, and the range of pathogenicity in experimental animals. Some of the more important characteristics are summarized in Table 28–1. Increasingly, this classification system is yielding to molecular-based techniques. The identification of species-specific rRNA and DNA sequences has resulted in the revision and expansion of the older phenotype-based classification system, and the provision of an increasing array of species-specific DNA probes to clinical mycobacteriology laboratories.

Distinguished by cultural features, biochemical reactions, and pathogenicity

# MYCOBACTERIAL DISEASE

Mycobacteria include a wide range of species pathogenic for humans and animals. Some, such as *M. tuberculosis,* occur exclusively in humans under natural conditions. Others, such as *Mycobacterium intracellulare,* can infect various hosts, including humans, but also exist in the free-living state. Most nonpathogenic species are widely distributed in the environment. Diseases caused by mycobacteria usually develop slowly, follow a chronic course, and elicit a granulomatous response. Infectivity of pathogenic species is quite high, but virulence for healthy humans is low. For example, disease following infection with *M. tuberculosis* is the exception rather than the rule.

Includes human and animal pathogens

Slowly progressive diseases

Mycobacteria do not produce classic exotoxins or endotoxins. Disease processes are thought to be the result of two related host responses. The first, a delayed-type hypersensitivity (DTH) reaction to mycobacterial proteins, results in the destruction of nonactivated macrophages containing multiplying organisms. It is detected by intradermal injections of purified proteins from the mycobacteria. The second, cell-mediated immunity (CMI) activates macrophages, enabling them to destroy mycobacteria contained

Lack exotoxins or endotoxins

TABLE 28–1

## Mycobacteria of Major Clinical Importance[a]

| Species | Characteristics: Reservoir | Virulence for Humans | Disease Caused | Case-to-Case Transmission | Growth Rate[b] | Optimum Growth Temperature | Pigment Production[c] | Substantial Niacin Production[d] | Virulence for Guinea Pigs[e] |
|---|---|---|---|---|---|---|---|---|---|
| *M. tuberculosis* | Human | +++ | Tuberculosis | Yes | S | 37 | – | + | + |
| *M. bovis* | Animals | +++ | Tuberculosis | Rare | S | 37 | – | – | + |
| Bacillus Calmette–Guérin | Artificial culture | ± | Local lesion | Very rare | S | 37 | – | – | – |
| *M. kansasii* | Environmental | + | Tuberculosis-like | No | S | 37 | Photochromogen | – | – |
| *M. scrofulaceum* | Environmental | + | Usually lymphadenitis | No | S | 37 | Scotochromogen | – | – |
| *M. avium-intracellulare* | Environmental; birds | + | Tuberculosis-like | No | S | 37 | ± | – | – |
| *M. fortuitum* | Environmental | ± | Local abscess | No | F | 37 | ± | – | Local abscess |
| *M. marinum* | Water; fish | ± | Skin granuloma | No | S | 30 | Photochromogen | – | – |
| *M. ulcerans* | Probably environmental; tropical | + | Severe skin ulceration | No | S | 30 | – | – | – |
| *M. leprae* | Human | +++ | Leprosy | Yes | NG | NG | NG | NG | – |
| *M. smegmatis* | Human, external urethral area | – | None | – | F | 37 | – | – | – |

[a] Numerous nonpathogenic environmental mycobacteria exist and may contaminate human specimens.

[b] S = slow (colonies usually develop in 10 days or more); F = fast (colonies develop in 7 days or less); NG = not grown.

[c] Yellow–orange pigment. Photochromogen is pigment produced in light; scotochromogen is pigment produced in dark or light.

[d] Many other differential biochemical tests used, eg, nitrate reduction, catalase production, Tween 80 hydrolysis.

[e] Disease following subcutaneous injection of light inoculum (eg, $10^2$ cells).

within their cytoplasm. The balance between these two responses determines the pathology and clinical response to a mycobacterial infection.

## MYCOBACTERIUM TUBERCULOSIS

### BACTERIOLOGY

*M. tuberculosis* is a slim, strongly acid–alcohol–fast rod. It frequently shows irregular beading in its staining, appearing as connected series of acid-fast granules (Fig 28–1). It grows at 37°C but not at room temperature, and it requires enriched or complex media for primary growth. Growth is enhanced by 5 to 10% carbon dioxide but is still very slow, with a mean generation time of 12 to 24 hours. The classic medium, Löwenstein–Jensen, contains homogenized egg in nutrient base with dyes to inhibit the growth of nonmycobacterial contaminants. The dry, rough, buff-colored colonies usually appear after 3 to 6 weeks of incubation. Mycobacterial growth is more rapid in two semisynthetic oleic acid–albumin media. Virulent strains grown in the latter demonstrate "cording" in which multiplying organisms remain attached in parallel bundles to form long intertwining cords or ropes. The major phenotypic tests for identification of *M. tuberculosis* are summarized in Table 28–1. Of particular importance is the ability of *M. tuberculosis* to produce large quantities of niacin, which is uncommon in other mycobacteria.

Growth requires rich medium and $CO_2$

Cording, biochemical tests distinguish from other mycobacteria

Due to its hydrophobic lipid surface, *M. tuberculosis* is unusually resistant to drying, to most common disinfectants, and to acids and alkalis. Tubercle bacilli are sensitive to heat, including pasteurization, and individual organisms in droplet nuclei are susceptible to inactivation by ultraviolet light.

Unusual resistance to drying and disinfectants but not to heat

As with other mycobacteria, the *M. tuberculosis* cell wall structure is dominated by mycolic acids and LAM. Its antigenic makeup includes many protein and polysaccharide antigens of which tuberculin is the most studied. It consists of heat-stable proteins liberated into liquid culture media. A purified protein derivative (PPD) of tuberculin is used for skin testing for hypersensitivity and is standardized in tuberculin units according to skin test activity.

PPD contains mix of tuberculin proteins

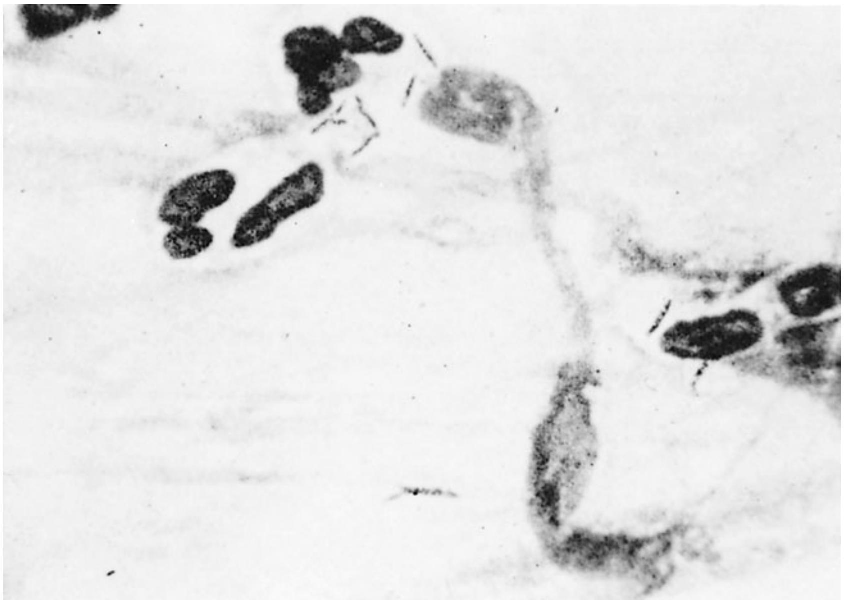

FIGURE 28–1
*Mycobacterium tuberculosis* in sputum stained by Ziehl–Neelsen technique. The mycobacteria retain the red carbol fuchsin through the decolorization step. The cells, background, and any other organisms stain with methylene blue counterstain.

# TUBERCULOSIS

CLINICAL CAPSULE

Tuberculosis is a systemic infection manifested only by evidence of an immune response in most exposed individuals. In some infected persons, the disease either progresses or, more commonly, reactivates after an asymptomatic period (years). The most common reactivation form is a chronic pneumonia with fever, cough, bloody sputum, and weight loss. Spread outside of the lung also occurs and is particularly devastating when it reaches the central nervous system. The natural history follows a course of chronic wasting to death aptly called "consumption" in the past.

## EPIDEMIOLOGY

A recognized disease of antiquity, tuberculosis first reached epidemic proportions in the western world during the major periods of urbanization in the 18th and 19th centuries. Mortality reached 200 to 700 per 100,000 population each year, accounting for 20 to 30% of all deaths in urban centers and winning tuberculosis the appellation of the "white plague." Morbidity was many times higher. The disease has had major sociologic components, flourishing with ignorance, poverty, overcrowding, and poor hygiene, particularly during the social disruptions of war and economic depression. Under these conditions, the poor are the major victims, but all sectors of society are at risk. Chopin, Paganini, Rousseau, Goethe, Chekhov, Thoreau, Keats, Elizabeth Barrett Browning, and the Brontës, to name but a few, were all lost to tuberculosis in their intellectual prime. With knowledge of the cause and transmission of the disease and the development of effective antimicrobial agents, tuberculosis was increasingly brought under control in developed countries. Unfortunately, mortality and morbidity remain at 19th-century levels in many developing countries despite extensive national and international control programs.

Infection of 18th and 19th centuries

Attack rates still high in many developing countries

The great majority of tuberculous infections are contracted by inhalation of droplet nuclei carrying the causative organism. Humans may also be infected through the gastrointestinal tract following the ingestion of milk from tuberculous cows (now uncommon due to pasteurization) or, rarely, through abraded skin. It has been estimated that a single cough can generate as many as 3000 infected droplet nuclei and that less than 10 bacilli may initiate a pulmonary infection in a susceptible individual. The likelihood of acquiring infection thus relates to the numbers of organisms in the sputum of an open case of the disease, the frequency and efficiency of the coughs, the closeness of contact, and the adequacy of ventilation in the contact area. Epidemiologic data indicate that large doses or prolonged exposure to smaller infecting doses is usually needed to initiate infection in humans. In some closed environments, such as a submarine or a crowded nursing home, a single open case of pulmonary tuberculosis can infect the majority of nonimmune individuals sharing sleeping accommodations.

Most infections are by respiratory route

Repeated coughing generates infectious dose into air

Poor ventilation increases risk

In the past, an animal variant (*Mycobacterium bovis*) was transmitted by drinking milk from infected herds. This disease has been largely eliminated by eradication programs and milk pasteurization.

The decline in mortality and occurrence of the disease in the United States over the last century is shown in Figure 28–2. Between 1953 to 1985 the number of new tuberculosis cases per annum fell from 84,304 to 22,201. By the mid-1980s, it was estimated that only 4 to 5% of American citizens, and less than 1% of American children, demonstrated positive tuberculin skin tests. However, the decline was not uniform throughout the American populace, and case rates among nonwhites and the urban poor remained significantly higher than the national average. As the incidence of infection in the United States and other developed countries decreased, there was also a major shift in the age of tuberculosis patients. Most were over 50 years of age and represented cases in which an old primary lesion, quiescent for decades, became reactivated. The grandfather who has developed "chronic bronchitis" is a classic source of infection to children.

Overall decline masks increases in some subpopulations

Reactivation among older persons

In 1985, the steady decline in reports of new tuberculosis cases and deaths in the United States ceased, and, in the ensuing 7 years, new cases increased by nearly 20%.

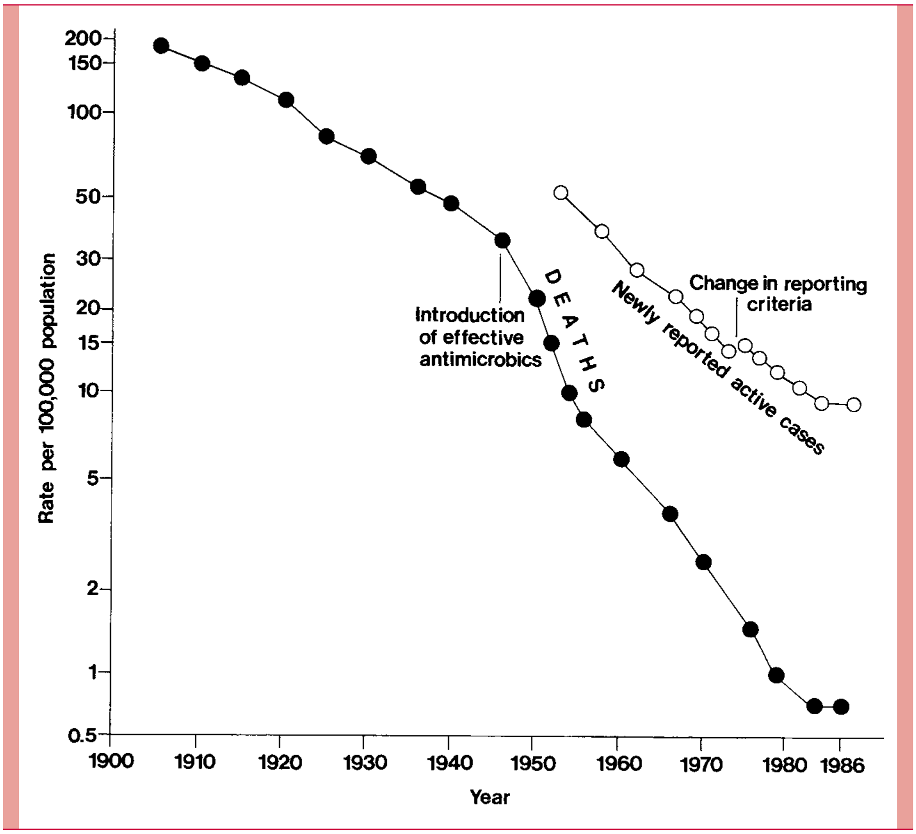


FIGURE 28–2
Morbidity and mortality of tuberculosis in the United States, 1900–1986.

Immigrants, impoverished, homeless, AIDS patients, and drug abusers

This change has been attributed to a significant decrease in the funding for tuberculosis control programs; spread of multiresistant strains of *M. tuberculosis;* increased immigration from tuberculosis-endemic areas of the world; social and economic changes that contributed to a burgeoning of incarcerated intravenous drug users and homeless populations; and, finally, to the AIDS epidemic. It is estimated that patients with latent tuberculosis increase their risk of reactivation disease by factors of 200 to 300 with the development of a human immunodeficiency virus (HIV) coinfection. The per annum reactivation rate of such individuals is estimated at 8%.

Rates increasing in children

Accompanying the increase in reactivation tuberculosis among high-risk US populations was an increase in the transmission of *M. tuberculosis*. Annual tuberculin skin conversions among intravenous drug abusers and the number of cases of tuberculosis among children under 5 years of age increased significantly between 1987 and 1990. Single-source epidemics involve school children and a teacher with unrecognized pulmonary tuberculosis, homeless shelters, nursing homes, and medical personnel exposed to patients with unrecognized tuberculosis. Since 1992, a reinvigorated public health effort in the United States has, again, led to a declining number of individuals with active tuberculosis, reaching a new low of 18,361 in 1998. This represents a rate of 6.8/100,000 population, still well short of the nation's interim goal of 3.5/100,000 for the year 2000 and 1/100,000 for the year 2010.

Resistance to antimicrobics increasing

Globally, the situation is more ominous. It is estimated that one third of the world's population is infected with *M. tuberculosis;* 30 million people have active disease, an additional 8 million develop new disease yearly, and 2 to 3 million die annually of this "captain of death." As a result, tuberculosis is the leading cause of death from an infectious disease worldwide. It is thought responsible for 6% of all deaths and 26% of avoidable adult deaths. Particularly concerning for the future control of tuberculosis worldwide is the marked susceptibility of patients with AIDS and the growing resistance of *M. tuberculosis* to the currently available antimicrobic agents. Because 40% of all new cases of tuberculosis in the United States are among foreign-born individuals, the elimination of this disease in the United States will be impossible without a substantial reduction in the global burden of tuberculosis.

## PATHOGENESIS

### Primary Infection

Primary tuberculosis is the response to the initial infection in an individual not previously infected and sensitized to tuberculoprotein. Inhaled droplet nuclei containing small numbers of tubercle bacilli are deposited in the peripheral respiratory alveoli, most frequently those of the well-ventilated middle and lower lobes. Here they are engulfed by **nonspecifically activated** alveolar macrophages. The ability of these cells to destroy ingested organisms depends significantly on their inherent microbicidal capacity. If the alveolar macrophages are unable to destroy ingested mycobacteria, they continue to multiply until the macrophage bursts. The released organisms are subsequently ingested by inactivated blood macrophages that, together with T cells, are attracted to the lung by chemotactic factors.

Inhaled organisms multiply in alveolar macrophages

The ingested mycobacteria continue to multiply intracellularly without damage to their host cell. Some of the bacterial-laden macrophages are transported through lymphatic channels to the hilar lymph nodes draining the infected site. From there, they may disseminate through blood and lymphatic systems to a number of tissues, including the liver, spleen, kidney, bone, brain, meninges, and apices or other parts of the lung. The inflammatory reaction in the seeded tissues is usually minor, and the signs and symptoms of infection are absent. However, the primary site of infection and some enlarged hilar lymph nodes can often be detected radiologically. In infants and immunocompromised adults, hematogenous dissemination of organisms may occasionally produce a life-threatening meningitis.

Low reactivity to the organism allows multiplication and dissemination to lymph nodes and bloodstream

Morphologically, the resulting tubercle is a microscopic granuloma comprised of some multinucleated giant cells formed by the fusion of several macrophages (Langhans cells), many epithelioid cells (activated macrophages), and a surrounding collar of lymphocytes (Fig 28–3) and fibroblasts. When many bacteria are present and there is a high degree of hypersensitivity, enzymes, reactive oxygen intermediates, and reactive nitrogen intermediates are released by dying macrophages and lead to necrosis of the center of the granuloma, which is termed caseous because of the cheesy, semisolid character of the gross lesion.

The tubercle includes activated macrophages and other cell types

Caseation occurs with high levels of antigen and hypersensitivity

Primary infections are usually handled well by the host. Bacterial multiplication ceases. Most microscopic lesions heal by fibrosis, and the organisms in them slowly die. In others, especially those in well-oxygenated tissues such as the subapical areas of the lung, renal

Organisms remain viable for long periods

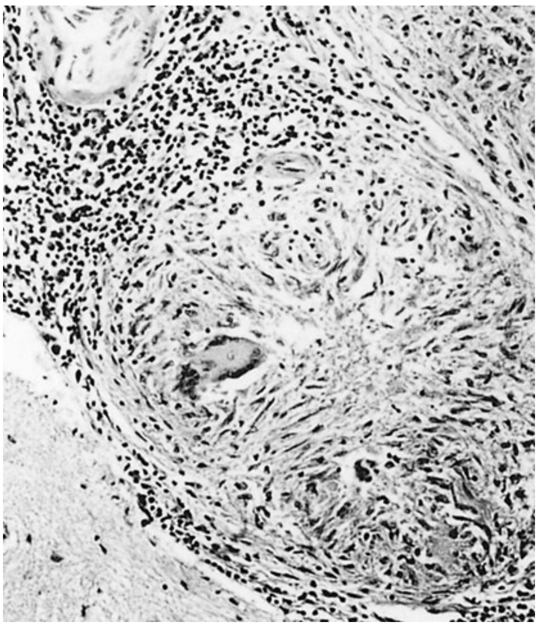

FIGURE 28–3
Microscopic tubercule of brain, showing giant cell and surrounding epithelioid cells and lymphocytes.

cortex and vertebral bodies, the tubercle bacilli remain viable for long periods and serve as a potential source of reactivation many months or years later if host defenses weaken.

## Reactivation (Adult) Tuberculosis

Discharge of caseous material forms pulmonary cavities

Reactivation usually occurs in body areas of relatively high oxygen tension and low lymphatic drainage, most often in the apex of the lung. The lesions show spreading, coalescing tubercles with numerous tubercle bacilli, and large areas of caseous necrosis. Necrosis often involves the wall of a small bronchus from which the necrotic material is discharged, resulting in a pulmonary cavity and bronchial spread. Frequently, small blood vessels are also eroded. The chronic fever and weight loss may be mediated in part by macrophage-derived tumor necrosis factor.

## Virulence Mechanisms

Ability to multiply in macrophages is central to virulence

Lipids modulate cytokines and inhibit killing

The basis for *M. tuberculosis* virulence is largely unknown. It produces no exotoxins, and both the intact cell and cellular components are remarkably innocuous to humans and experimental animals not previously sensitized to tuberculin. Cell wall components such as LAM have been implicated in binding to alveolar macrophages, utilizing surface fibronectin, mannose, or complement receptors (CR1, CR3). Once inside, multiple factors contribute to survival and continued multiplication. A number of genes have been identified that are linked to virulence by enhancing survival in the macrophage or by influencing the physical and chemical conditions (low pH, high lactic acid, high $CO_2$) present in developing lesions, but their function remains unknown. Mycolic acids, sulfolipids, LAM, and proteins have been shown to disrupt phagosome–lysosome interactions and interfere with oxidative killing. LAM has also been shown to modulate cytokine production and downregulate other aspects of T-cell function including antigen presentation.

# IMMUNITY

Innate immunity is high and genetically variable

Humans generally have a rather high innate immunity to development of disease. This was tragically illustrated in the Lübeck disaster of 1926 where infants were administered *M. tuberculosis* instead of an intended vaccine strain. Despite the large dose, only 76 of 249 died and most of the others developed only minor lesions. Approximately 10% of immunocompetent persons infected with *M. tuberculosis* will develop active disease any time in their life. There is epidemiologic and historic evidence for differences in the immunity in certain population groups and between identical and nonidentical twins.

DTH and CMI develop in 2–6 weeks

Mycobacterial antigens are presented by infected macrophages

Cytokines mediate destruction and further inflammation

DTH to tuberculoprotein and CMI to *M. tuberculosis* develop 2 to 6 weeks after primary infection. The subsequent course of the infection depends on the balance between these two defensive mechanisms. DTH, through the mediation of natural killer cells, destroys the inactivated macrophages as well as the surrounding tissues, releasing still viable mycobacteria into an area of necrosis unsuitable for bacterial multiplication. CMI develops when competent T lymphocytes recognize mycobacterial antigen complexes on the surface of *M. tuberculosis*–containing macrophages. In the presence of macrophage-produced interleukin-1, the activated lymphocytes respond to the presented antigens with the elaboration of several cytokines. Some of these proteins attract circulating monocytes. Others, including interferon-$\gamma$ and possibly tumor necrosis factor-$\alpha$, activate local tissue macrophages and the recruited monocytes to enhanced destruction of ingested mycobacteria, resulting in a slowing or discontinuation of intracellular bacterial growth. Nitrous oxide or other reactive nitrogen intermediates probably mediate the destruction of the mycobacteria. Another cytokine, interleukin-2, induces clonal expansion of the activated lymphocytes, thus amplifying the host's immunologic response. Still others stimulate accumulation of fibroblasts and deposition of collagen, which help wall off the area of infection and prevent further dissemination.

Immunity is cell mediated but incomplete

Acquired immunity is cell mediated but incomplete. Both helper–inducer (CD4+) and cytotoxic (CD8+) T lymphocytes are involved. Two to three weeks after infection, macrophages are activated at the site of infection by a network of pro- and anti-inflammatory cytokines and chemokines from antigen-stimulated CD4+ T lymphocytes, macrophages, and dendritic cells. This interaction between *M. tuberculosis* and the host is what eventually limits its multiplication and spread. Cytotoxic T cells release bacilli from inactivated phagocytic cells and allow them to be ingested and handled by the activated macrophages. The concomitant DTH to tuberculoprotein plays an important part in immunity to reinfection by mobilizing immune cells and macrophages to the site of deposition of tubercle bacilli. In the past, it was believed that reinfection from external sources was extremely rare, but it is now clear that loss of hypersensitivity and CMI can occur over time and that reinfection can develop into clinical tuberculosis.

CD4+ and CD8+ T lymphocytes are involved

DTH enhances immunity to reinfection

The role of DTH in immunity of established tuberculosis is complex, because high degrees of sensitivity can precipitate caseous necrosis and lead to spread of the disease. The importance of CMI and hypersensitivity in modulating the course of tuberculosis is, perhaps, most dramatically illustrated in patients with AIDS. Those with minimal impairment of cellular immune responses develop typical tubercles containing relatively few bacilli. Those with advanced impairment demonstrate abundant acid-fast bacilli without epithelioid cell accumulation or associated tissue necrosis.

Hypersensitivity can precipitate caseation and spread in established disease

Lesions in AIDS patients related to degree of immunosuppression

# TUBERCULOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

### Primary Tuberculosis

Primary tuberculosis is either asymptomatic or manifest only by fever and malaise. Radiographs may show infiltrates in the mid-zones of the lung and enlarged draining lymph nodes in the area around the hilum. When these lymph nodes fibrose and sometimes calcify, they produce a characteristic picture (Ghon complex) on radiograph. In approximately 5% of patients, the primary disease is not controlled and merges into the reactivation type of tuberculosis, or it disseminates to many organs to produce active miliary tuberculosis. The latter may result from a necrotic tubercle eroding into a small blood vessel.

Mid-lung infiltrates and adenopathy are produced

Primary infection may progress to reactivation or miliary tuberculosis

### Reactivation Tuberculosis

Approximately 10% of those recovering from a primary infection develop clinical disease sometime during their lifetime. In Western countries, reactivation of previous quiescent lesions occurs most often after the age of 50 and is more common in men. Reactivation is associated with a period of immunosuppression precipitated by malnutrition, alcoholism, diabetes, old age, and a dramatic change in the individual's life, such as loss of a spouse. In areas in which the disease is more common, reactivation tuberculosis is more frequently seen in young adults experiencing the immunosuppression that accompanies puberty and pregnancy. Recently, reactivation and progressive primary tuberculosis among younger adults have increased as a complication of AIDS.

Reactivation is most common in older men

Predisposing factors include underlying disease and life events

Cough is the universal symptom. It is initially dry, but as the disease progresses sputum is produced, which even later is mixed with blood (hemoptysis). Fever, malaise, fatigue, sweating, and weight loss all progress with continuing disease. Radiographically, infiltrates appearing in the apices of the lung coalesce to form cavities with progressive destruction of lung tissue. Less commonly, reactivation tuberculosis can also occur in other organs, such as the kidneys, bones, lymph nodes, brain, meninges, bone marrow, and bowel. Disease at these sites ranges from a localized tumor-like granuloma (tuberculoma) to a fatal chronic meningitis. Untreated, the progressive cough, fever, and weight loss of pulmonary tuberculosis creates an internally consuming fire that usually takes 2 to 5 years to cause death. The course in AIDS and other CMI-compromised patients is more rapid.

Cough is universal

Cavities form in lung apices

Multiple organs are involved

## DIAGNOSIS

### Tuberculin Test

The tuberculin skin test measures DTH to tuberculoprotein. PPD is standardized biologically against an international reference preparation, and its activity expressed in

PPD test measures hypersensitivity to tuberculoprotein

tuberculin units (TU). Most initial skin tests employ 5 TU (intermediate strength). When an unusually high degree of hypersensitivity or eye or skin tuberculosis is suspected, then 1 TU (first strength) or less is used initially to avoid the risk of an excessive reaction locally or at the site of a mycobacterial lesion.

PPD test interpreted by area of induration

Positive PPD indicates past or current infection

The test most commonly performed involves intradermal injection that is read 48 to 72 hours later. An area of measured induration of 10 mm or more accompanied by erythema constitutes a positive reaction, although smaller areas of induration and erythema indicate a lesser degree of sensitization to mycobacterial proteins. No induration indicates a negative reaction. A positive PPD test indicates that the individual has been infected at some time with *M. tuberculosis* or with a strongly cross-reacting mycobacterium of another species. It carries no implication about the activity of the infection, which may have been simply a primary complex contracted 20 years previously.

Anergy may develop with therapy or disease affecting CMI

A negative PPD test in a healthy individual indicates that he or she has not been infected with *M. tuberculosis,* is in the prehypersensitive stage of a primary infection, or has finally lost tuberculin sensitivity along with disappearance of antigen from an old primary complex. Patients with severe disseminated disease, those on steroid or immunosuppressive drugs, or those with certain other diseases such as AIDS and measles, may also become anergic. They lose their tuberculin hypersensitivity and become more susceptible to the disease. Induration below the 10-mm diameter criterion for positivity indicates low-level sensitization, which may be attributable to *M. tuberculosis* infection or to a cross-reacting mycobacterial infection.

Clinical value of skin test depends on prevalence of reactivity in population

The clinical value of the PPD test depends on the occurrence of primary infection in different age groups. Now, primary infection is sufficiently uncommon in much of the Western world that a negative test is frequently important in excluding tuberculosis. A positive test in infancy or childhood has significance in diagnosis and can often be used to trace a household or school source of infection. Epidemiologic surveys of tuberculin reactivity indicate trends in the incidence of infection and constitute the simplest way of monitoring the effectiveness of control measures.

## Laboratory Diagnosis

Mycobacteria are detected in direct smears of clinical material

Contaminating mycobacteria may yield "false" positives in some specimens

If present in sufficient numbers, acid-fast bacilli can be detected microscopically in direct smears of clinical specimens or in smears of material concentrated for culture (see below). Smears are stained by the Ziehl–Neelsen procedure or one of its modifications, including the fluorescence staining method. About 65% of culture-positive sputum samples yield positive smears from concentrated specimens. These procedures are not specific for *M. tuberculosis* because other mycobacteria may have a similar morphology and may be etiologic agents of disease, members of the normal flora, or external contaminants. Their significance depends on the specimen. Acid-fast bacilli in sputum are highly significant for mycobacterial infection. A clean-voided male urine specimen, on the other hand, is often contaminated with *Mycobacterium smegmatis* from the prepuce, and the finding of acid-fast bacilli does not per se indicate infection. Bronchoscopy equipment and nasotracheal tubes or their lubricants are prone to contamination with free-living mycobacteria, and false conclusions have been drawn from smears of such preparations. The polymerase chain reaction has been reported to be useful in the direct diagnosis of tuberculosis by a number of investigators. To date, none of these techniques are practical for routine use in the clinical laboratory.

Material contaminated with normal flora must be treated

Mucolytic agents used in sputum

NaOH used as antibacterial

Cultural confirmation of a tentative diagnosis of tuberculosis is thus essential, and the organism must be isolated for identification and susceptibility testing. Specimens from protected sites, such as cerebrospinal fluid, bone marrow, pleural fluid, and ureteric urine, can be seeded directly to culture media used for *M. tuberculosis* isolation. Those samples inevitably contaminated with normal flora, such as sputum, gastric aspirations (cultured when sputum is not available, for example, in young children), or voided urine, are treated with alkali, acid, or a detergent germicide under conditions that kill the normal flora but allow many mycobacteria to survive because of their resistance to these agents. The most commonly used treatment now employs *N*-acetylcysteine to dissolve mucus, combined with the antibacterial effect of a weak sodium hydroxide solution. The material is concentrated by centrifugation or filtration, neutralized or washed, and inoculated onto culture media.

Cultures on solid media usually take 3 weeks or longer to show visible colonies. Growth may be detected radiometrically in about half the time by using liquid oleic acid–albumin broth containing $^{14}C$-labeled palmitic acid, which is metabolized by mycobacteria to liberate $^{14}CO_2$. The labeled $CO_2$ is detected in the space above the medium using an automated sampling procedure. Incorporation of a specific inhibitor of *M. tuberculosis* in a parallel vial increases the specificity of the test.

Traditional cultures take 3+ weeks; labeled substrate procedures are twice as fast

Whichever procedure is used, specific identification of an isolated mycobacterium is essential. It may be achieved with a number of cultural and biochemical tests, including those shown in Table 28–1, but the process usually takes several weeks. More rapid results can be obtained by high-resolution gas chromatographic analysis of fatty acids in mycobacterial colonies or by testing for homology between genetic probes of labeled mycobacterial DNA and ribosomal RNA extracted from the strain under test. Specific probes are now available commercially for detecting *M. tuberculosis* and the *Mycobacterium avium–intracellulare* complex.

Traditional speciation uses cultural and biochemical tests

DNA/RNA homology is useful

Susceptibility testing is important with newly diagnosed cases. When sufficient numbers of acid-fast bacilli are seen on direct smears, the treated clinical specimen can be seeded directly onto antimicrobic-containing media for susceptibility tests, thereby saving several weeks. If numbers are scanty, the initiation of tests must await primary isolation. More rapid test results can be obtained by incorporating antimicrobics into the medium used for radiometric detection of mycobacterial growth. These results show good concordance with conventional tests and are available 1 to 2 weeks earlier.

Susceptibility testing by conventional and labeled substrate procedures

## TREATMENT

*M. tuberculosis* is susceptible to several effective antimicrobics (Table 28–2). Isoniazid, ethambutol, rifampin, pyrazinamide, streptomycin, and combinations of these agents constitute the primary drugs of choice for treatment of tuberculosis. All of these, except ethambutol, are bactericidal. Isoniazid and rifampin are active against both intra- and extracellular organisms, and pyrazinamide, a nicotinamide analog, acts at the acidic pH found within cells. Streptomycin does not penetrate into cells and is thus active only against extracellular organisms. *M. tuberculosis* is also susceptible to other drugs that may be used to replace those of the primary group if they are inappropriate because of resistance or drug toxicity. The fluoroquinolones, such as ciprofloxacin and ofloxacin, are active against *M. tuberculosis* and penetrate well into infected cells. Their role in the treatment of tuberculosis is under evaluation. Isoniazid and ethambutol act on the mycolic acid (isoniazid) and LAM (ethambutol) elements of mycobacterial cell wall synthesis. The molecular targets of the other agents have yet to be defined except for the general antibacterial agents (rifampin, streptomycin, fluoroquinolones) discussed in Chapter 13.

Multiple antimicrobics act intra- and extracellularly

Resistance or toxicity may limit some agents

TABLE 28–2

**Antimicrobics Commonly Used in Treatment of Tuberculosis**

| First-Line Drug | Second-Line Drug[a] |
|---|---|
| Isoniazid | *para*-Aminosalicylic acid |
| Ethambutol | Ethionamide |
| Rifampin | Cycloserine |
| Pyrazinamide | Fluoroquinolones |
| Streptomycin | Kanamycin, etc |

[a] Second-line drugs added to combinations if resistance or toxicity contraindicates first-line agent.

Mutational resistance to antituberculous drugs occurs at frequencies of $10^{-7}$ to $10^{-10}$. For example, mutation in a gene coding for a catalase-peroxidase enzyme causes failure of the conversion of isoniazid to its biologically active form. Such mutants often come to predominate and produce clinical relapse particularly when a single drug is used. Adequate, continuous treatment with two or three antituberculous drugs with different modes of action greatly reduces the probability a mutant will be expressed, because the chance of a doubly resistant mutant in a lesion's organism population is very low. The proportion of infections with strains resistant to first-line drugs varies between 5 and 15% but appears to be increasing in many locales, particularly among individuals who have been treated previously. Of particular concern is the establishment in the last decade of strains resistant to both isoniazid and rifampin, the mainstays of primary treatment. Susceptibility tests are required to guide drug selection.

Combined therapy used to prevent resistance

Treatment with multiple antimicrobics to which the organism is susceptible usually renders the patient noninfectious within 1 or 2 weeks, which has shifted the care of tuberculous patients from isolation hospitals and sanatoriums to the home or the general hospital. After an initial intense phase of systemic chemotherapy, treatment is usually continued with oral antimicrobics for several months. Until recently, therapy with two oral agents, isoniazid and ethambutol, was continued for a total of 18 to 24 months. Studies have now demonstrated that therapy can be shortened to 9 months when isoniazid and rifampin are used concomitantly and to 6 months when pyrazinamide is added as a third agent. In patients whose organisms display resistance to one or more of these drugs, and in those with HIV infection, a more prolonged treatment course is used. The effectiveness of chemotherapy on most forms of tuberculosis has been dramatic and has greatly reduced the need for surgical procedures such as pulmonary lobectomy. Failure of chemotherapy is often associated with lack of adherence to the regimen by the patient, the presence of resistant organisms, or both.

Even "short" courses of treatment last 9 months

Resistance and HIV infection require lengthier treatment

Compliance a major problem

## PREVENTION

Prophylactic chemotherapy, usually with isoniazid alone, is now used in situations in which known or suspected primary tuberculous infection poses the risk of clinical disease. Some indications for prophylaxis are summarized in Table 28–3. Isoniazid can be used alone in prophylaxis because the load of tubercle bacilli in a subclinical primary lesion is small in relation to that in reactivation tuberculosis, and experience has shown that the development of subsequent clinical disease from isoniazid-resistant strains selected by prophylaxis can be discounted. Unfortunately, isoniazid may cause a form of hepatitis, and the risk increases progressively after 20 years of age. Its use in older subjects involves balancing risk against potential benefit and requires monitoring with liver function tests.

Chemoprophylaxis may use single drug

At present, the bacillus Calmette-Guérin (BCG) vaccine (named for its originators, Calmette and Guérin) is the only available vaccine. It has been used for prophylaxis of tuberculosis in various countries since 1923; administration is usually intradermal. It is a live vaccine derived originally from a strain of *M. bovis* that was attenuated by repeated subculture. Since then, it has had a checkered history, with results in different controlled trials ranging from ineffectiveness to 80% protection. In most studies, however, it has substantially decreased the highly lethal miliary and meningeal forms of tuberculosis among young children. On the basis of these results, massive immunization campaigns sponsored by the World Health Organization have been organized in underdeveloped countries.

BCG vaccine is a live attenuated derivative of *M. bovis*

Effectiveness of BCG is variable

BCG is used only in tuberculin-negative subjects. Successful vaccination leads to a minor local lesion, self-limiting multiplication of the organism locally and in draining lymphatic vessels, and development of tuberculin hypersensitivity. The latter results in loss of the PPD test as a diagnostic and epidemiologic tool, and when infection rates are low, as they are now in most Western countries, this loss may offset the possible immunity produced. In general, tuberculosis rates in the West have declined as rapidly in countries that have not used the BCG vaccine as in those that have adopted mass vaccination with its occasional complications. Its potential value in these countries is restricted to population groups at particular risk. Its role in developing countries remains a matter of some

PPD conversion caused by BCG

BCG contraindicated for AIDS patients

contention. The BCG vaccination is contraindicated for individuals in whom cell-mediated immune mechanisms are compromised, such as those infected with the HIV.

## *MYCOBACTERIUM LEPRAE*

### BACTERIOLOGY

*Mycobacterium leprae,* the cause of leprosy, is an acid-fast bacillus that has not been grown in artificial medium or tissue culture beyond, possibly, a few generations. However, it can be grown in the footpads of normal mice, in thymectomized irradiated mice, and in the armadillo, which may also be infected naturally. Its growth in animals is very slow, with an estimated doubling time of 12 to 14 days. Although lack of in vitro growth severely limits study of the organism, the structure and cell wall components appear to be similar to other mycobacteria. One mycoside, phenolic glycolipid I (PGL-1), is synthesized in large amounts and found only in *M. leprae*.

Fails to grow in culture

Slow growth in animals

### LEPROSY

CLINICAL CAPSULE

Leprosy is a chronic granulomatous disease of the peripheral nerves and superficial tissues, particularly the nasal mucosa. Disease ranges from slowly resolving anesthetic skin lesions to the disfiguring facial lesions responsible for the social stigma and ostracism of the individuals with leprosy (lepers).

#### EPIDEMIOLOGY

The exact mode of transmission is unknown but appears to be by generation of small droplets from the nasal secretions from cases of lepromatous leprosy. Traumatic inoculation through minor skin lesions or tattoos is also possible. The central reservoir is infected humans. The incubation period as estimated from clinical observations is generally 2 to 7 years but sometimes up to four decades. Very rarely, cases develop in nonendemic areas without known case contacts. The infectivity of *M. leprae* is low. Most new cases have had prolonged close contact with an infected individual. Biting insects may also be involved. Although virtually absent from North America and Europe, there are still an estimated 10 million infected persons in Asia, Africa, and Latin America. Immigration into Western countries from areas where the disease occurs has increased the numbers of cases seen.

Nasal droplets transmit infection

Rare in North America

#### PATHOGENESIS

*M. leprae* is an obligate intracellular parasite that must multiply in host cells to persist. In humans the preferred cells are macrophages and Schwann cells. PGL-1 and LAM have been implicated in the ability to survive and multiply in these cells. The organism may invade peripheral sensory nerves, resulting in patchy anesthesia. Few *M. leprae* are seen in tuberculoid lesions, which are granulomatous with extensive epithelioid cells, giant cells, and lymphocytic infiltration. In lepromatous multibacillary leprosy, CMI is deficient, and growth of *M. leprae* is, thus, relatively unimpeded. Histologically, lesions show dense infiltration with leprosy bacilli, and large numbers may reach the bloodstream.

Obligate intracellular parasite of macrophages and Schwann cells

#### IMMUNITY

Immunity to *M. leprae* is CMI mediated. The range of disease correlates with DTH responsiveness to lepromin, a skin test antigen derived from leprous tissue similar to tuberculin.

CMI determines extent of disease

Tuberculoid cases have minimal disease and positive skin tests. Lepromatous cases have progressive disease and negative skin tests. Other tests of CMI response to *M. leprae* correlate in the same way.

# LEPROSY: CLINICAL ASPECTS

## MANIFESTATIONS

Two major forms of the disease are recognized, tuberculoid and lepromatous. However, intermediate forms occur, and the first form may merge into the second.

### Tuberculoid Leprosy

Skin and nerve involvement

Strong delayed hypersensitivity and CMI

Tuberculoid leprosy involves the development of macules or large, flattened plaques on the face, trunk, and limbs, with raised, erythematous edges and dry, pale, hairless centers. When the bacterium has invaded peripheral nerves, the lesions are anesthetic. The disease is indolent, with simultaneous evidence of slow progression and healing. Because of the small number of organisms present, this form of the disease is usually noncontagious.

### Lepromatous Leprosy

Deficient CMI and anergy to lepromin

Many *M. leprae* in lesions

In lepromatous multibacillary leprosy, CMI is deficient, and patients are anergic to lepromin. Growth of *M. leprae* is, thus, relatively unimpeded. Histologically, lesions show dense infiltration with leprosy bacilli, and large numbers may reach the bloodstream. Skin lesions are extensive, symmetric, and diffuse, particularly on the face, with thickening of the looser skin of the lips, forehead, and ears, resulting in the classic leonine appearance. Damage may be severe, with loss of nasal bones and septum, sometimes of digits, and with testicular atrophy in men. The organism spreads systemically, with involvement of the reticuloendothelial system.

## DIAGNOSIS

Acid-fast smears and biopsies are primary diagnostic methods

Laboratory diagnosis of lepromatous leprosy involves preparation of acid-fast stained scrapings of infected tissue, particularly nasal mucosa or ear lobes. Large numbers of acid-fast bacilli are seen. Tuberculoid leprosy is diagnosed clinically and by histologic appearance of full-thickness skin biopsies. PGL-1–based serologic tests have been evaluated for their usefulness in serodiagnosis. The specificity has been excellent, but the sensitivity for tuberculoid leprosy is still unsatisfactory. It is likely that suitable serologic tests will be available for this disease in the near future.

## TREATMENT AND PREVENTION

Sulfones combined with rifampin primary treatment

Prevention requires early diagnosis and treatment of cases

Treatment has been revolutionized by the development of sulfones, such as dapsone, which blocks *para*-aminobenzoic acid metabolism in *M. leprae*. When combined with rifampin, dapsone usually controls or cures tuberculoid leprosy when given for 6 months. In lepromatous leprosy and multibacillary intermediate forms of the disease, a third agent (clofazimine) is added to help prevent the selection of resistant mutants, and treatment is continued at least 2 years. Prevention of leprosy involves recognition and treatment of infectious patients and early diagnosis of the disease in close contacts. Chemoprophylaxis with sulfones has been used for children in close contact with lepromatous cases. Immunization with BCG vaccine has been investigated, with varying results.

A possible diagnosis of leprosy elicits fear and distress in patients and contacts out of all proportion to its risks. Few clinicians in the United States have the experience to make such a diagnosis, and expert help should be sought from public health authorities before reaching this conclusion or indicating its possibility to the patient.

## MYCOBACTERIA CAUSING TUBERCULOSIS-LIKE DISEASES

Mycobacteria causing diseases that often resemble tuberculosis are listed in Table 28–1. With the exception of *M. bovis,* they have become relatively more prominent as the incidence of tuberculosis has declined. All have known or suspected environmental reservoirs, and all the infections they cause appear to be acquired from these sources. Immunocompromised individuals or those with chronic pulmonary conditions or malignancies are more likely to develop disease. There is no evidence of case-to-case transmission. The organisms grow on the same media as *M. tuberculosis* but usually more rapidly. Colonies of some species produce yellow or orange pigment in the light (photochromogenic), and some in the light and dark (scotochromogenic). Species are distinguished by these characteristics and by biochemical reactions. Environmental mycobacteria that cause tuberculosis-like infections are usually more resistant than *M. tuberculosis* to some of the antimicrobics used in the treatment of mycobacterial diseases, and susceptibility testing is often needed as a guide to therapy.

Acquired from the environment; no case-to-case transmission

Some species are pigmented

Resistance common

### *Mycobacterium kansasii*

*Mycobacterium kansasii* is a photochromogenic mycobacterium that usually forms yellow-pigmented colonies after about 2 weeks of incubation in the presence of light. In the United States, infection is most common in Illinois, Oklahoma, and Texas and tends to affect urban residents; it is uncommon in the Southeast. There is no evidence of case-to-case transmission, but the reservoir has yet to be identified. It causes about 3% of mycobacterial disease in the United States.

*M. kansasii* infections resemble tuberculosis and tend to be slowly progressive without treatment. Cavitary pulmonary disease, cervical lymphadenitis, and skin infections are most common, but disseminated infections also occur. They are an important cause of disease in patients with HIV infection and CD4+ T lymphocyte counts of less than 200 cells/$\mu$L; clinical features closely resemble tuberculosis in patients with AIDS. Hypersensitivity to proteins of *M. kansasii* develops and cross-reacts almost completely with that caused by tuberculosis. Positive PPD tests may thus result from clinical or subclinical *M. kansasii* infection. Prolonged combined chemotherapy with isoniazid, rifampin, and ethambutol is usually effective.

Resembles tuberculosis

Infection may cause PPD conversion

### *Mycobacterium avium–Intracellulare* Complex

*Mycobacterium avium–intracellulare* complex is a group of related acid-fast organisms that grow only slightly faster than *M. tuberculosis* and can be divided into a number of serotypes. Among them are organisms that cause tuberculosis in birds (and sometimes swine) but rarely lead to disease in humans. Others may produce disease in mammals, including humans, but not in birds. They are found worldwide in soil and water and in infected animals. In the United States they are most common in the Southeast, Pacific Coast, and north central regions. They are second only to *M. tuberculosis* in significance and frequency of the diseases they cause.

*M. avium-intracellulare* complex associated with birds and mammals

Second only to *M. tuberculosis* as cause of disease in United States

The most common infection in humans is cavitary pulmonary disease, often superimposed on chronic bronchitis and emphysema. Most individuals infected are white men of 50 years of age or more. Cervical lymphadenitis, chronic osteomyelitis, and renal and skin infections also occur. The organisms in this group are substantially more resistant to antituberculous drugs than most other species, and treatment with the three or four agents

Wide range of diseases; most common are pulmonary

Relative resistance to antituberculous drugs found to be most active often requires supplementation with surgery. About 20% of cases relapse within 5 years of treatment.

Disseminated *M. avium–intracellulare* infections, once considered rare, are now the most common systemic bacterial infection in patients with AIDS. They usually develop when the patient's general clinical condition and CD4+ helper T lymphocyte concentrations are declining. Clinically, the patient experiences progressive weight loss and intermittent fever, chills, night sweats, and diarrhea. Histologically, granuloma formation is muted, and there are aggregates of foamy macrophages containing numerous intracellular acid-fast bacilli. The diagnosis is most readily made by blood culture, using a variety of specialized cultural techniques. Identification can be rapidly accomplished with the use of specific DNA probes. Response to chemotherapeutic agents is marginal, and the prognosis is grave.

Disseminated infection is a common complication of AIDS

Organisms isolated from blood

### *Mycobacterium scrofulaceum*

*Mycobacterium scrofulaceum* is an acid-fast scotochromogen that occurs in the environment under moist conditions. It forms yellow colonies in the dark or light within 2 weeks, and it shares several features with the *M. avium–intracellulare* complex. *Mycobacterium scrofulaceum* is now one of the more common causes of granulomatous cervical lymphadenitis in young children. It derives its name from scrofula, an old descriptive term for tuberculous cervical lymphadenitis. The infection manifests as an indolent enlargement of one or more lymph nodes with little, if any, pain or constitutional signs. It may ulcerate or form a draining sinus to the surface. It does not cause PPD conversion. Treatment usually involves surgical excision.

Granulomatous cervical lymphadenitis in children

## MYCOBACTERIAL SOFT TISSUE INFECTIONS

### *Mycobacterium fortuitum* Complex

*Mycobacterium fortuitum* complex comprises free-living, rapidly growing, acid-fast bacilli that produce colonies within 3 days. Human infections are rare. Abscesses at injection sites in drug abusers are probably the most common lesions. Occasional secondary pulmonary infections develop. Some cases have been associated with implantation of foreign material (eg, breast prostheses, artificial heart valves). Except in the case of endocarditis, infections usually resolve spontaneously with removal of the prosthetic device.

Rapid growers cause abscesses and infections of prostheses

### *Mycobacterium marinum*

*Mycobacterium marinum* causes tuberculosis in fish, is widely present in fresh and salt waters, and grows at 30°C but not at 37°C. It occurs in considerable numbers in the slime that forms on rocks or on rough walls of swimming pools and thrives in tropical fish aquariums. It can cause skin lesions in humans. Classically, a swimmer who abrades his or her elbows or forearms climbing out of a pool develops a superficial granulomatous lesion that finally ulcerates. It usually heals spontaneously after a few weeks but is sometimes chronic. The organism may be sensitive to tetracyclines as well as to some antituberculous drugs.

Cause of fish tuberculosis

### *Mycobacterium ulcerans*

*Mycobacterium ulcerans* is a much more serious cause of superficial infection. (Like *M. marinum, M. ulcerans* grows at 30°C but not at 37°C [see Table 28–1].) Cases usually occur in the tropics, most often in parts of Africa, New Guinea, and northern Australia, but have been seen elsewhere sporadically. Children are most often affected. The source of infection and mode of transmission are unknown. Infected individuals develop severe ulceration involving the skin and subcutaneous tissue that is often progressive unless

Occurs in tropical areas

Severe, progressive ulcerations require surgical removal treated effectively. Surgical excision and grafting are usually needed. Antimicrobic treatment is often unsuccessful.

## ADDITIONAL READING

Advisory Council for the Elimination of Tuberculosis. Tuberculosis elimination revisited: Obstacles, opportunities, and a renewed commitment. *MMWR Morb Mortal Wkly Rep* 1999; 48(RR09);1–13. Review of the current status of tuberculosis in the United States, recognizing that additional diagnostic, therapeutic, and immunization tools will have to be developed to eradicate this disease.

Barnes PF, Bloch AB, Davidson PT, Snider DE Jr. Tuberculosis in patients with human immunodeficiency virus infection. *N Engl J Med* 1991;324:1644–1650. A recent review of the impact of one of humankind's newest scourges on one of its oldest.

Daniel TM. Antibody and antigen detection in the immunodiagnosis of tuberculosis. Why not? What more is needed? Where do we stand today? *J Infect Dis* 1988;158:678–680.

Dubos RJ, Dubos J. *The White Plague. Tuberculosis, Man, and Society*. Boston: Little, Brown; 1952. A scholarly and highly readable account of the history and impact of tuberculosis on Western culture.

Falkinham JO Jr. Epidemiology of infection by nontuberculous mycobacterium. *Clin Microbiol Rev* 1996;9:177–215.

Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW. The emergence of drug-resistant tuberculosis in New York City. *N Engl J Med* 1993;328:521–526. A frightening look at the future.

Gaylord H, Brennan PJ. Leprosy and the leprosy bacillus. Recent developments in characterization of antigens and immunology of the disease. *Annu Rev Microbiol* 1987;41: 645–675.

Hastings RC, Gillis TP, Krahenbuhl JL, et al. Leprosy. *Clin Microbiol Rev* 1988; 1:330–348. The preceding two references are recent comprehensive reviews of this biblical disease, with an emphasis on its microbiology and immunology.

Interlied CB, Kemper CA, Bermudez LEM. The *Mycobacterium avium* complex. *Clin Microbiol Rev* 1993;6:266–310. A comprehensive review of all aspects of this increasingly important group including its role in AIDS patients.

Riley RL, Mills CC, O'Grady F, et al. Infectiousness of air from a tuberculosis ward. Ultraviolet irradiation of infected air: Comparative infectiousness of different patients. *Am Rev Resp Dis* 1962;85:511–525. This paper is the last in a series of "classic" studies exploring factors related to the aerial dissemination of tuberculosis. They demonstrated that infectivity varied greatly in different patients with similar pulmonary diseases, and that it decreased rapidly with the onset of chemotherapy. They also established that a patient with laryngeal tuberculosis was significantly more infectious than patients with cavitary pulmonary disease.

Schlossberg D (ed). *Tuberculosis and Nontuberculous Mycobacterial Infections,* 4th ed. Philadelphia: WB Saunders; 1999. A recent, excellent monograph covering all aspects of mycobacterial pathophysiology, pathogenesis, epidemiology, clinical manifestations, diagnosis, and treatment of mycobacterial infections.

Sepkowitz KA. How contagious is tuberculosis? *Clin Infect Dis* 1996;23:954–962. The author reviews and updates information on the contagiousness of pulmonary tuberculosis. This, in conjunction with Riley's articles, provides the most definitive information available.

Slutsker L, Castro KG, Ward JW, Dooley SW Jr. Epidemiology of extrapulmonary tuberculosis among persons with AIDS in the United States. *Clin Infect Dis* 1993; 16:513–518.

Tuberculosis Progress Report. *Lancet* 1999;353:995–1006. A compendium of articles on the worldwide status of tuberculosis, the impact of AIDS on its spread, and the implication of increasing antimicrobic resistance on its control.

van Crevel R, Ottenhoff THM, van der Meer JWM. Innate immunity to *Mycobacterium tuberculosis*. *Clin Microbiol Rev* 2002;15: 294–309. This review examines all aspects of the immune response to *M. tuberculosis* with particular emphasis on the roles of cytokines and chemokines.

Wolinsky E. Mycobacterial disease other than tuberculosis. *Clin Infect Dis* 1992; 15:1–12. A recent highly readable and comprehensive summary of the mycobacteria that have been termed “atypical” and of the clinical diseases they produce.

CHAPTER 29

# *Actinomyces* and *Nocardia*

KENNETH J. RYAN

*Actinomyces* and *Nocardia* are Gram-positive rods characterized by filamentous, tree-like branching growth, which has caused them to be confused with fungi in the past. They are opportunists that can sometimes produce indolent, slowly progressive diseases. A related genus, *Streptomyces,* is of medical importance as a producer of many antibiotics, but it rarely causes infections. Important differential features of these groups and of the mycobacteria to which they are related are shown in Table 29–1.

## ACTINOMYCES

### BACTERIOLOGY

*Actinomyces* are Gram-positive bacilli that grow slowly (4–10 days) under microaerophilic or strictly anaerobic conditions. The organisms typically appear as elongated Gram-positive rods that branch at acute angles and often show irregular staining. In pus the most characteristic form is the sulfur granule. This yellow–orange granule, named for its gross resemblance to a grain of sulfur, is a small colony (usually <0.3 mm) of intertwined branching *Actinomyces* filaments solidified with elements of tissue exudate.

Slow-growing anaerobic branching Gram-positive rods

Species of *Actinomyces* are distinguished on the basis of biochemical reactions, cultural features, and cell wall composition. Most human actinomycosis is caused by *Actinomyces israelii,* but other species have been isolated from typical actinomycotic lesions. *Propionibacterium propionicum* originally classified with the *Actinomyces,* can produce clinically similar disease. Other species of *Actinomyces* have been associated with dental and periodontal infections (see Chapter 62).

Most infections due to *A. israelii*

### ACTINOMYCOSIS

CLINICAL CAPSULE

Actinomycosis is a chronic inflammatory condition originating in the tissues adjacent to mucosal surfaces. The lesions follow a slow burrowing course with considerable induration and draining sinuses eventually opening through the skin. The exact nature depends on the organs and structures involved.

TABLE 29–1

**Features of Actinomycetes**

| Genus | Morphology | Acid Fastness | Growth | Source | Disease |
|---|---|---|---|---|---|
| *Actinomyces* | Branching bacilli | None | Anaerobic | Oral, intestinal endogenous flora | Chronic cellulitis, draining sinuses |
| *Nocardia* | Branching bacilli | Weak[a,b] | Aerobic | Soil | Pneumonia, skin pustules, brain abscess |
| *Rhodococcus* | Cocci to bacilli | Variable (weak[a]) | Aerobic | Soil, horses[c] | Pneumonia |
| *Streptomyces* | Branching bacilli | None | Aerobic | Soil | Extremely rare[d] |

[a] Modified stain, fast only to weak decolorizer (1% $H_2SO_4$).

[b] *N. asteroides* and *N. brasiliensis*; other species variable.

[c] *R. equi*.

[d] Nonpathogen but important producer of antibiotics.

Normal flora throughout gastrointestinal tract

Conditions for growth require displacement into tissues

Sinus tracts contain pus and sulfur granules

*Actinomyces* are normal inhabitants of some areas of the gastrointestinal tract of humans and animals from the oropharynx to the lower bowel. These species are highly adapted to mucosal surfaces and do not produce disease unless they transgress the epithelial barrier under conditions that produce a sufficiently low oxygen tension for their multiplication. Such conditions usually involve mechanical disruption of the mucosa with necrosis of deeper, normally sterile tissues (eg, following tooth extraction). Once initiated, growth occurs in microcolonies in the tissues and extends without regard to anatomic boundaries. The lesion is composed of inflammatory sinuses, which ultimately discharge to the surface. As the lesion enlarges, it becomes firm and indurated. Sulfur granules are present within the pus but are not numerous. Free *Actinomyces* or small branching units are rarely seen, although contaminating Gram-negative rods are common. As with other anaerobic infections (see Chapter 19) most cases are polymicrobial involving other flora from the mucosal site of origin.

No evidence of immunity

Human cases provide little evidence of immunity to *Actinomyces*. Once established, infections typically become chronic and resolve only with the aid of antimicrobic therapy. Antibodies can be detected in the course of infection but seem to reflect the antigenic stimulation of the ongoing infection rather than immunity. Infections with *Actinomyces* are endogenous, and case-to-case transmission does not appear to occur.

## ACTINOMYCOSIS: CLINICAL ASPECTS

### MANIFESTATIONS

Cervicofacial forms are linked to dental hygiene

Actinomycosis exists in several forms that differ according to the original site and circumstances of tissue invasion. Infection of the cervicofacial area, the most common site of actinomycosis (Fig 29–1), is usually related to poor dental hygiene, tooth extraction, or some other trauma to the mouth or jaw. Lesions in the submandibular region and the angle of the jaw give the face a swollen, indurated appearance.

Surgery, trauma, and intrauterine devices provide opportunity

Thoracic and abdominal actinomycoses are rare and follow aspiration or traumatic (including surgical) introduction of infected material leading to erosion through the pleura, chest, or abdominal wall. Diagnosis is usually delayed, because only vague or nonspecific symptoms are produced until a vital organ is eroded or obstructed. The firm, fibrous masses are often initially mistaken for a malignancy. Pelvic involvement as an extension from other sites also occurs occasionally. It is particularly difficult to distinguish

FIGURE 29–1
Cervicofacial actinomycosis. Note the "lumpy jaw" swelling and the draining sinuses at the angle of the jaw.

from other inflammatory conditions or malignancies. A more localized chronic endometritis, apparently caused by *Actinomyces*, has been associated with the use of intrauterine contraceptive devices.

## DIAGNOSIS

A clinical diagnosis of actinomycosis is based on the nature of the lesion, the slowly progressive course, and a history of trauma or of a condition predisposing to mucosal invasion by *Actinomyces*. The etiologic diagnosis can be difficult to establish with certainty. Although the lesions may be extensive, the organisms in pus may be few and concentrated in sulfur granule microcolonies deep in the indurated tissue. The diagnosis is further complicated by heavy colonization of the moist draining sinuses with other bacteria, usually Gram-negative rods. This contamination not only causes confusion regarding the etiology but interferes with isolation of the slow-growing anaerobic *Actinomyces*. Material for direct smear and culture should include as much pus as possible to increase the chance of collecting the diagnostic sulfur granules.

Sinus drainage contains few *Actinomyces*

Drainage is often contaminated with other species

Sulfur granules crushed and stained show a dense, Gram-positive center with individual branching rods at the periphery (Fig 29–2). Granules should also be selected for culture, because material randomly taken from a draining sinus usually grows only superficial contaminants. Culture media and techniques are the same as those used for other anaerobes (see Chapters 15 and 19). Incubation must be prolonged, because some strains require 7 days or more to appear. Identification requires a variety of biochemical tests to differentiate *Actinomyces* from propionibacteria (anaerobic diphtheroids), which may show a tendency to form short branches in fluid culture.

Gram stains show branching rods

Anaerobic culture is required

Biopsies for culture and histopathology are useful, but it may be necessary to examine many sections and pieces of tissue before sulfur granule colonies of *Actinomyces* are found. The morphology of the sulfur granule in tissue is quite characteristic with routine hematoxylin and eosin (HE) or histologic Gram staining. With HE, the edge of the granule shows amorphous eosinophilic "clubs" formed from the tissue elements and containing the branching actinomycotic filaments.

Biopsy shows characteristic clubbed lesions

## TREATMENT

Penicillin G is the treatment of choice for actinomycosis, although a number of other antimicrobics (tetracycline, erythromycin, clindamycin) are active in vitro and have shown some clinical effectiveness. High doses of penicillin must be used and therapy prolonged for 4 to 6 weeks or longer before any response is seen. Although slow, response to therapy is often striking given the degree of fibrosis and deformity caused by the infection. Because detection of the causative organism is difficult, many patients are treated empirically as a therapeutic trial based on clinical findings alone.

Penicillin may have to be used empirically

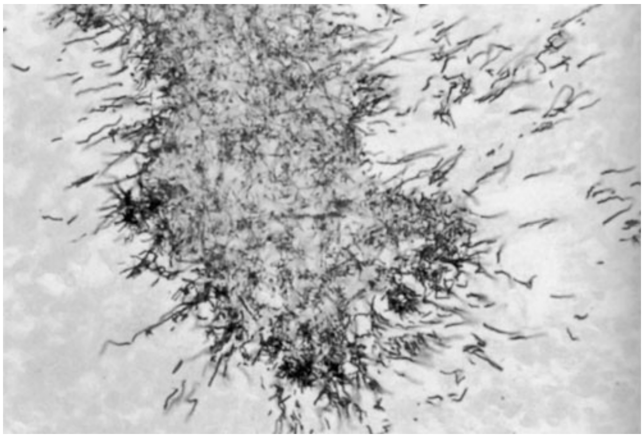

FIGURE 29–2
Sulfur granule. The bacteria are clearly seen to be Gram-positive and branching only at the edge.

# *Nocardia*

## BACTERIOLOGY

Beaded, branching Gram-positive rods are weakly acid fast

*Nocardia* species are Gram-positive, rod-shaped bacteria that show true branching both in culture and in stains from clinical lesions. The microscopic morphology is similar to that of *Actinomyces,* although *Nocardia* tend to fragment more readily and are found as shorter branched units throughout the lesion rather than concentrated in a few colonies or granules. Many strains take the Gram stain poorly, appearing "beaded" with alternating Gram-positive and Gram-negative sections of the same filament (Fig 29–3). The species most common in human infection (*N. asteroides* and *N. brasiliensis*) are weakly acid fast.

Grow on common media in 2–3 days

In contrast to *Actinomyces, Nocardia* species are strict aerobes. Growth typically appears on ordinary laboratory medium (blood agar) after 2 to 3 days incubation in air. Colonies initially have a dry, wrinkled, chalk-like appearance, are adherent to the agar, and eventually develop white to orange pigment. Speciation involves uncommon tests such as the decomposition amino acids and casein.

## NOCARDIOSIS

CLINICAL CAPSULE

Nocardiosis occurs in two major forms. The pulmonary form is an acute bronchopneumonia with dyspnea, cough and sputum production. A cutaneous form produces localized pustules in areas of traumatic inoculation usually the exposed areas of the skin.

### EPIDEMIOLOGY

Primary source is soil

Occurrence in the immunocompromised is increased

*Nocardia* species are ubiquitous in the environment, particularly in soil. In fact, fully developed colonies of *Nocardia* give off the aroma of wet dirt. The organisms have been isolated in small numbers from the respiratory tract of healthy persons, but are not considered members of the normal flora. The pulmonary form of disease follows inhalation of aerosolized bacteria, and the cutaneous form follows injection by a thorn prick or similar accident. The majority of pulmonary cases occur in patients with compromised immune systems due to underlying disease or the use of immunosuppressive therapy.

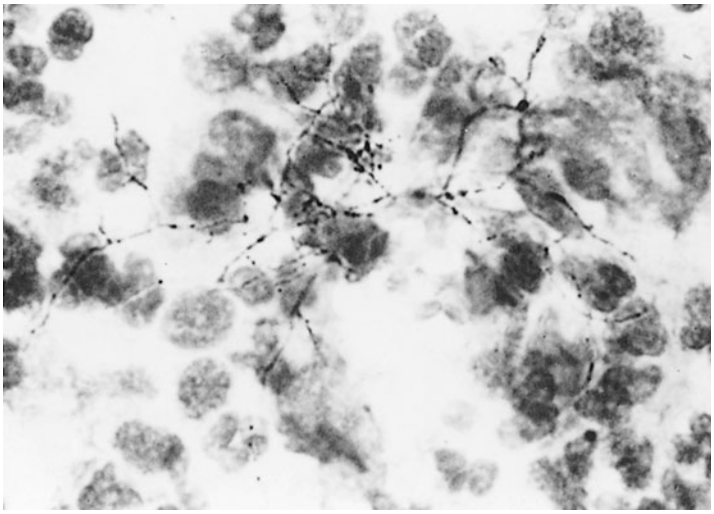

FIGURE 29–3
*Nocardia* in sputum. Note the filamentous bacteria forming treelike branches among the neutrophils. The beaded appearance of the rods is typical. (*Reprinted with permission of Schering Corporation, Kenilworth, NJ, the copyright owner. All rights reserved.*)

Transplant patients have been a prominent representative of the latter group. There is no case-to-case transmission.

## PATHOGENESIS

Factors leading to disease following inhalation of *Nocardia* are poorly understood. Neutrophils are prominent in nocardial lesions but appear to be relatively ineffective. The bacteria have the ability to resist the microbicidal actions of phagocytes and may be related to disruption of phagosome acidification or resistance to the oxidative burst. No specific virulence factors are known. The primary lesions in the lung show acute inflammation, with suppuration and destruction of parenchyma. Multiple, confluent abscesses may occur. Unlike *Actinomyces* infections, there is little tendency toward fibrosis and localization. Dissemination to distant organs, particularly the brain, may occur. In the central nervous system (CNS), multifocal abscesses are often produced. The great majority of *Nocardia* pulmonary and brain infections are produced by *N. asteroides.*

Able to survive in phagocytes

Pulmonary infection is usually *N. asteroides*

CNS dissemination produces abscesses

Skin infections follow direct inoculation of *Nocardia*. This mechanism is usually associated with some kind of outdoor activity and with relatively minor trauma. The species is usually *N. brasiliensis*, which produces a superficial pustule at the site of inoculation. If *Nocardia* gain access to the subcutaneous tissues, lesions resembling actinomycosis may be produced, complete with draining sinuses and sulfur granules. This infection may occur with *Nocardia* species or related organisms such as *Actinomadura madurae* (formerly *Nocardia madurae*), a cause of the mycetoma syndrome (see Chapter 47).

Cutaneous infections follow minor trauma

## IMMUNITY

There is evidence that effective T cell–mediated immunity is dominant in host defense against *Nocardia* infection. Increased resistance to experimental *Nocardia* infection in animals has been mediated by cytokine-activated macrophages, and activated macrophages have enhanced capacity to kill *Nocardia* that they have engulfed. Patients with impaired cell-mediated immune responses are at greatest risk for nocardiosis. There is little evidence for effective humoral immune responses.

Cell-mediated immunity mechanisms are dominant

# NOCARDIOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

Pulmonary infection is usually a confluent bronchopneumonia that may be acute, chronic, or relapsing. Production of cavities and extension to the pleura are common. Symptoms

Bronchopneumonia and cerebral abscess findings depend on localization

are those of any bronchopneumonia, including cough, dyspnea, and fever. The clinical signs of brain abscess depend on its exact location and size; the neurologic picture can be particularly confusing when multiple lesions are present. The combination of current or recent pneumonia and focal CNS signs is suggestive of *Nocardia* infection. The cutaneous syndrome typically involves a pustule, fever, and tender lymphadenitis in the regional lymph nodes.

## DIAGNOSIS

Gram stain is usually positive

Weak acid fastness is characteristic

Blood agar is sufficient for culture

The diagnosis of *Nocardia* infection is much easier than that of actinomycosis, because the organisms are present in greater numbers throughout the lesions. Filaments of Gram-positive rods with primary and secondary branches can usually be found in sputum and are readily demonstrated in direct aspirates from skin or other purulent sites. Demonstration of acid-fastness, when combined with other observations, is diagnostic of *N. asteroides* or *N. brasiliensis*. The acid-fastness of *Nocardia* species is not as strong as that of mycobacteria. The staining method thus employs a decolorizing agent weaker than that used for the classic stain. Culture of *Nocardia* is not difficult, because the organisms grow on blood agar. It is still important to alert the laboratory to the possibility of nocardiosis, because the slow growth of *Nocardia* could cause it to be overgrown by the respiratory flora commonly found in sputum specimens. Specific identification can take weeks due the unconventional tests involved.

## TREATMENT

Sulfonamides are active

*Nocardia* are usually susceptible to sulfonamide, but relatively resistant to penicillin. The trimethoprim–sulfamethoxazole combination is the most widely used chemotherapeutic regimen. Technical difficulties in susceptibility testing have hampered the rational selection and study of other antimicrobics, but various reports support clinical activity of newer $\beta$-lactams (imipenem, ceftriaxone), minocycline, and aminoglycosides. Antituberculous agents and antifungal agents such as amphotericin B have no activity against *Nocardia*.

# *Rhodococcus*

Morphology varies from cocci to rods

Pneumonia is associated with horses

*Rhodococcus* is a genus of aerobic actinomycetes with characteristics similar to those of *Nocardia.* Morphologically the rods vary from cocci to long, curved, clubbed forms. Some strains are acid-fast. *Rhodococcus* has recently been recognized as an opportunistic pathogen causing an aggressive pneumonia in severely immunocompromised patients, particularly those with acquired immunodeficiency syndrome. The organisms are found in the soil. One species, *Rhodococcus equi,* has an association with horses where it also causes pneumonia in foals. This species is a facultative intracellular pathogen of macrophages with features somewhat similar to those of *Legionella* and *Listeria*. Optimal treatment is unknown, although erythromycin, aminoglycosides, and some $\beta$-lactams show in vitro activity.

## ADDITIONAL READING

Lerner PI. Nocardiosis. *Clin Infect Dis* 1996;22:891–905.

Smego RA, Foglia G. Actinomycosis. *Clin Infect Dis* 1998;26:1255–1263.

Both these reviews are part of the "State-of-the-Art Clinical Article" series. They consider pathogenesis as well as clinical aspects and are well referenced.

CHAPTER 30

# Chlamydia

W. LAWRENCE DREW

Members of the genus *Chlamydia* are obligate intracellular bacteria, which have all the elements of bacteria except a rigid cell wall. Of the three species causing disease in humans, *Chlamydia trachomatis* is the most common as a major cause of genital infection and conjunctivitis. A chronic form of *C. trachomatis* conjunctivitis, called trachoma, is the leading preventable cause of blindness in the world. *Chlamydia pneumoniae* and *Chlamydia psittaci* are respiratory pathogens. Our knowledge of biology and pathogenesis of these bacteria is based primarily on the study of *C. trachomatis*.

## *CHLAMYDIA TRACHOMATIS*

## BACTERIOLOGY

### MORPHOLOGY

*C. trachomatis* are round cells between 0.3 and 1 $\mu$m in diameter depending on the replicative stage (see below). The envelope surrounding the cells includes a trilaminar outer membrane that contains lipopolysaccharide and proteins similar to those of Gram-negative bacteria. A major difference is that chlamydiae lack the thin peptidoglycan layer between the two membranes. They are obligate intracellular parasites and have not been grown outside eukaryotic cells. The genome of is one of the smallest among prokaryotes and lacks genes for amino acid synthesis. *C. trachomatis* has ribosomes and is able to carry out the common energy producing pathways of other bacteria.

Envelope has no peptidoglycan layer between membranes

Obligate intracellular bacteria, which fail to grow in artificial media

DNA homology between *C. trachomatis, C. psittaci,* and *C. pneumoniae* is less than 30%, although rRNA sequence analysis suggests they share a common origin. The three species share a common group antigen. Their major differential features are shown in Table 30–1. Two biovars of *C. trachomatis* affect humans: trachoma and lymphogranuloma venereum (LGV). *C. trachomatis* has multiple outer membrane proteins that further divide the biovars into multiple serovars, or strains (Table 30–2).

### REPLICATIVE CYCLE

The replicative cycle of chlamydiae is illustrated in Figure 30–1. It involves two forms of the organism: a small, hardy infectious form termed the elementary body (EB), and a larger fragile intracellular replicative form termed the reticulate body (RB). The major difference

Elementary body enters epithelial cells by endocytosis

TABLE 30–1

**Major Differential Features of Chlamydia Species that Cause Human Disease**

| Feature | *C. trachomatis* | *C. psittaci* | *C. pneumoniae* |
|---|---|---|---|
| Natural host | Humans | Birds; also livestock, cats | Humans |
| Disease | Conjunctivitis, pneumonia (infants), genital tract infections, lymphogranuloma venereum | Pneumonia, endocarditis | Bronchitis, pneumonia, ?atherosclerosis |
| Glycogen-containing inclusion bodies | Yes | No | No |
| Sulfonamide susceptibility | Yes | No | No |

Host cell metabolism used for growth and replication

Transiently inhibits apoptosis of infected cells

between the EB and the RB is the extent of cross-linking of the major outer membrane protein (MOMP); EB proteins are highly linked by disulfide bonds, and RBs less so. The EB is a metabolically inert form which neither expends energy nor synthesizes protein. The cycle begins when the EB attaches to unknown receptors on the plasma membrane of susceptible target cells (usually columnar or transitional epithelial cells). It then enters the cell in an endocytotic vacuole and begins the process of converting to the replicative RB. There is evidence that pinocytosis may also occur. Endosomes containing *C. trachomatis* EBs maintain a near neutral pH and fuse with each other but not with lysosomes. As the RBs increase in number, the endosomal membrane expands by fusing with lipids of the Golgi apparatus eventually forming a large inclusion body. After 24 to 72 hours, the process reverses and the RBs reorganize and condense to yield multiple EBs. The endosomal membrane then either disintegrates or fuses with the host cell membrane, releasing the EBs to infect adjacent cells. The metabolic changes that lead the EB to reorganize into the larger reticulate body are incompletely understood, but involve protein synthesis and modification of MOMPs between the monomeric and cross-linked state. *C. trachomatis* also inhibits apoptosis of epithelial cells, thus enabling completion of its replicative cycle.

## *Chlamydia trachomatis* DISEASES

**Clinical Capsule**

Ocular trachoma, with progressive inflammation and scarring leading to blindness, has been recognized since antiquity, but the role of *Chlamydiae* in conjunctivitis and pneumonia in young infants, and in a variety of genital infections was only clarified during the past 40 years. Like trachoma, the genital infections can persist or recur, with chronic sequelae.

TABLE 30–2

**Epidemiologic Associations between Chlamydial Species, Serovars (Strains), and Diseases**

| Species | Serovars (Strains) | Modes of Transmission | Diseases |
|---|---|---|---|
| *C. trachomatis* | A,B,Ba,C | Hand to eye, fomites, flies | Trachoma |
| | B,Ba,D–K | Sexual, intrapartum, hand to eye | Inclusion conjunctivitis; genital infection |
| | $L_1$,$L_2$,$L_3$ | Sexual | Lymphogranuloma venereum |
| *C. psittaci* | Many | Aerosol | Psittacosis |
| *C. pneumoniae* | TWAR[a] | Human to human | Respiratory infection |

[a] TW and AR were the laboratory designations for the first conjunctival and respiratory isolates, respectively.

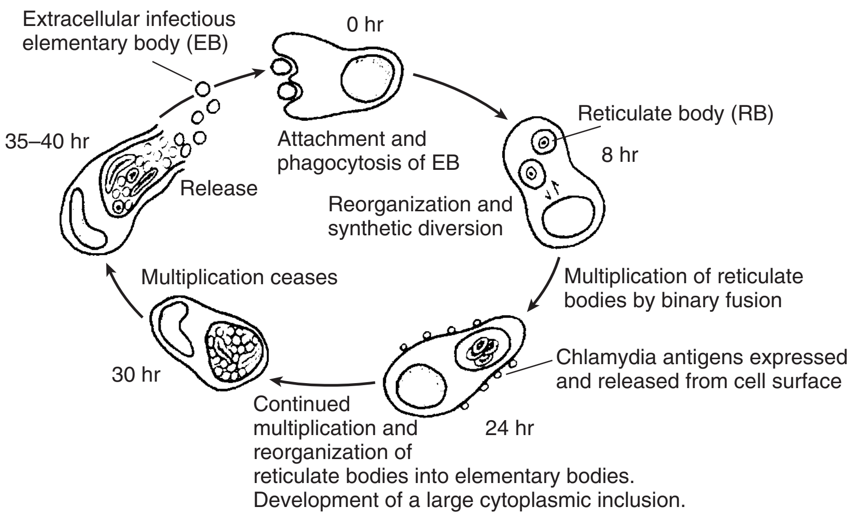


FIGURE 30–1
Reproduction cycle of *Chlamydia.*

## EPIDEMIOLOGY

*C. trachomatis* causes disease in several sites, including the conjunctiva and genital tract. It is spread by secretions and is the most common sexually transmitted disease. In the United States over 700,000 cases are reported each year, which is twice the number for gonorrhea. Humans are the sole reservoir (see Table 30–1). Each of the major disease syndromes caused by chlamydiae are associated with several different strains (see Table 30–2). Inclusion conjunctivitis is seen among population groups in which the strains causing *C. trachomatis* genital infections are common. This disease is the most common form of neonatal conjunctivitis in the United States, occurring in 2 to 6% of newborn infants. The infection results from direct contact with infective cervical secretions of the mother at delivery.

Neonatal conjunctivitis contracted from maternal genital infection

High attack rate

Trachoma, a chronic follicular conjunctivitis, afflicts an estimated 500 million persons worldwide and has blinded millions, particularly in Africa. The disease is usually contracted in infancy or early childhood from the mother or other close contacts. Spread is by contact with infective human secretions, directly via hands to the eye, or via fomites transmitted on the feet of flies.

Fomites, fingers, and flies involved in transmission of trachoma

The prevalence of chlamydial urethral infection in US men and women ranges from 5% in the general population to 20% in those attending sexually transmitted disease clinics. Approximately one third of male sexual contacts of women with *C. trachomatis* cervicitis develop urethritis after an incubation period of 2 to 6 weeks. The proportion of men with mild to absent symptoms is higher than in gonorrhea. Nongonococcal urethritis is most commonly caused by *C. trachomatis* and less frequently by *Ureaplasma urealyticum*. Reinfection is common.

High rate of sexual transmission

## PATHOGENESIS

Chlamydiae have a tropism for epithelial cells of the endocervix and upper genital tract of women, and the urethra, rectum and conjunctiva of both sexes. The LGV biovar can also enter through breaks in the skin or mucosa. Once infection is established, there is a release of proinflammatory cytokines such as interleukin-8 by infected epithelial cells. Chlamydial lipopolysaccharides probably also play an important role in initiation of the inflammatory process. This results in early tissue infiltration by polymorphonuclear leukocytes, later followed by lymphocytes, macrophages, plasma cells and eosinophils. If the infection progresses further (because of lack of treatment and/or failure of immune control), aggregates of lymphocytes and macrophages (lymphoid follicles) may form in the submucosa; these can progress to necrosis, followed by fibrosis and scarring.

Early release of proinflammatory cytokines

Later development of fibrosis and scarring

Persistent or recurrent infections cause chronic eye or genital sequelae

Autoimmunity may play an important role

The chronic sequelae of progressive inflammation with scarring that are seen in trachoma and some female genital tract infections are commonly due to persistent or recurrent infections, which may, in turn, be controlled by host cell immune responses. One theory is that this may result from molecular mimicry, involving epitopes found on the chlamydial 60-kd heat shock protein and also on human cells.

## IMMUNITY

Immunity is short-lived

Secretory IgA and CD4+ lymphocytes may influence severity

*C. trachomatis* infections do not reliably result in protection against reinfection although there is evidence that secretory immunoglobulin A may confer at least some partial immunity against genital tract reinfection. Any strain-specific protection that may result is short-lived. Local production of antibody, along with CD4+ lymphocytes of the Th1 type that traffic to the genital mucosa may together play a role in mitigating most acute infections. This would at least partially explain why most untreated chlamydial genital tract infections are persistent, but often subclinical in character.

# *Chlamydia trachomatis:* CLINICAL ASPECTS

## MANIFESTATIONS

### Eye Infections

Trachoma and inclusion conjunctivitis due to different serotypes

Trachoma and inclusion conjunctivitis are distinct diseases of the eye that have some overlap in their clinical manifestations. Trachoma, a chronic conjunctivitis caused by *C. trachomatis* strains A, B, Ba, and C, is usually seen in less developed countries and often leads to blindness. Inclusion conjunctivitis, an acute infection commonly caused by strains D to K, is usually not associated with chronicity or permanent eye damage. It occurs in newborns and adults worldwide.

#### *Trachoma*

Leading cause of blindness in some developing countries

Chronic inflammation of the eyelids and increased vascularization of the corneal conjunctiva are followed by severe corneal scarring and conjunctival deformities. Visual loss often occurs 15 to 20 years after the initial infection, due to repeated scarring of the cornea.

#### *Inclusion Conjunctivitis*

Smears or cultures from conjunctiva diagnostic

Inclusion conjunctivitis usually presents as an acute, copious, mucopurulent eye discharge 5 to 25 days after birth. Infection occurs in roughly two thirds of infants born vaginally to infected mothers, and one third of these become overtly ill. Inclusion conjunctivitis is clinically similar but less common in adults, and is usually associated with concomitant genital tract disease. Diagnosis can be made by demonstrating characteristic cytoplasmic inclusions in smears of conjunctival scrapings (Fig 30–2), by demonstration of antigen by direct immunofluorescence, or by culture from conjunctival swabs. Systemic therapy is preferred because the nasopharynx, rectum, and vagina may also be colonized and other forms of disease may develop, such as an infant pneumonia syndrome.

### Genital Infections

Clinical spectrum is similar to *N. gonorrhoeae*

The clinical spectrum of sexually transmitted infections with *C. trachomatis* is similar to that of *Neisseria gonorrhoeae*. *C. trachomatis* can cause urethritis and epididymitis in men and cervicitis, salpingitis, and a urethral syndrome in women. In addition, three strains of *C. trachomatis* cause LGV, another sexually transmitted disease (see Table 30–2).

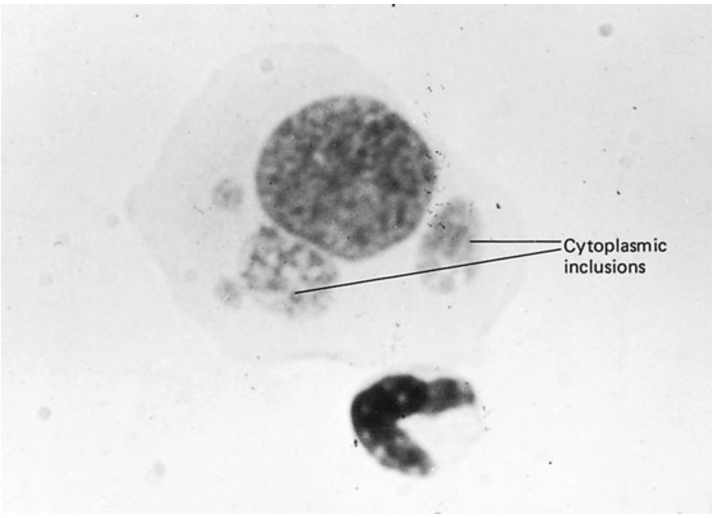


FIGURE 30–2
*Chlamydia trachomatis* cytoplasmic inclusion bodies in a conjunctival epithelial cell.

*C. trachomatis* urethritis is manifested by dysuria and a thin urethral discharge. Infections of the uterine cervix may produce vaginal discharge but are usually asymptomatic. Ascending infection in the form of salpingitis and pelvic inflammatory disease occurs in an estimated 5 to 30% of infected women. The scarring produced by chronic or repeated infection is an important cause of sterility and ectopic pregnancy.

Urethritis in men, but many asymptomatic

Salpingitis and pelvic inflammatory disease can cause permanent sequelae

More than 50% of all infants born to mothers excreting *C. trachomatis* during labor show evidence of infection during the first year of life. Most develop inclusion conjunctivitis (see earlier discussion), but 5 to 10% develop an infant pneumonia syndrome. *C. trachomatis* accounts for about one third to one half of all cases of interstitial pneumonia in infants. The illness usually develops between 6 weeks and 6 months of age and has a gradual onset. The child is usually afebrile, but develops difficulty in feeding, a characteristic staccato (pertussis-like) cough, and shortness of breath. The disease is rarely fatal but may be associated with decreased pulmonary function later in life.

Infant pneumonia syndrome has delayed, gradual onset

LGV is a sexually transmitted infection caused by *C. trachomatis* strains $L_1$, $L_2$, or $L_3$. It occurs principally in South America and Africa, although small outbreaks have recently occurred in North America. The clinical course is characterized by transient genital lesions followed by multilocular suppurative involvement of the inguinal lymph nodes. The primary genital lesion is usually a small painless ulcer or papule, which heals in a few days and may go unnoticed. The most common presenting complaint is inguinal adenopathy. Nodes are initially discrete, but as the disease progresses they become matted and suppurative (bubos). The skin over the node may be thinned, and multiple draining fistulas develop. Systemic symptoms such as fever, chills, headaches, arthralgia, and myalgia are common. Late complications include urethral or rectal strictures and perirectal abscesses and fistulas. In homosexual men, LGV strains can cause a hemorrhagic ulcerative proctitis. Lymph nodes may need to be aspirated to prevent rupture. A further consideration of genital tract infections is given in Chapter 70.

Papule and inguinal adenopathy

Abscesses, strictures, and fistulas with chronic infection

## DIAGNOSIS

All direct *C. trachomatis* diagnostic tests require the collection of epithelial cells from the site of infection. Inflammatory cells are not useful and should be cleaned away as much as possible. For genital infections, cervical specimens are preferred in females and urethral scrapings in males. Eye infections require conjunctival scrapings.

Epithelial cells are required for detection

Isolation of *C. trachomatis* has been the "gold standard" for diagnosis. It is achieved in cell culture using idoxuridine- or cycloheximide-treated McCoy cells. Treatment of the cells with antimetabolites inhibits host cell replication but allows chlamydiae to use available cell nutrients for growth. After inoculation with samples and incubation for 3 to 7 days, the cells

Isolation requires special treatment of cell lines

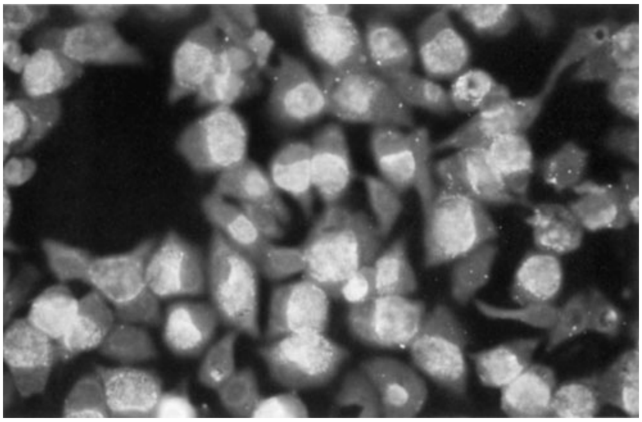

FIGURE 30–3
*Chlamydia trachomatis* cytoplasmic inclusions in tissue culture stained with fluorescein-labeled monoclonal antibodies. (*Reproduced with permission from Connor DH, Chandler FW, Schwartz DQA, Manz HJ, Lack EE (eds).* Pathology of Infectious Diseases, *vol. 1. Stamford, CT: Appleton & Lange; 1997.*)

*C. trachomatis* inclusions contain glycogen and are stainable with iodine

are stained with fluorescein-labeled monoclonal antibodies to detect intracytoplasmic chlamydiae (Fig 30–3). *C. trachomatis* reticulate bodies synthesize large amounts of glycogen, and the inclusion bodies in the cell thus stain reddish-brown with iodine. *C. psittaci* and *C. pneumoniae* inclusions do not contain glycogen.

LCR or PCR are most sensitive methods of noncultural diagnosis

A large number of procedures are now available for noncultural direct detection of *C. trachomatis* in clinical specimens. These include direct fluorescent antibody (DFA) methods using monoclonal antibodies directed against outer membrane proteins of elementary bodies in epithelial cells, enzyme immunoassays that detect chlamydial lipopolysaccharide, and assays for *C. trachomatis* DNA. These tests are faster, and, in the case of DNA assays, also more sensitive than culture. Ligase chain reaction (LCR) or polymerase chain reaction (PCR) are the most sensitive methods of diagnosis. The latter procedures can be used on urine samples, which are easier to obtain than cervical or urethral samples. This greatly aids the detection of chlamydial genital infections, especially in adolescents, and ultimately facilitates control of the spread of this sexually transmitted disease.

Serodiagnosis not helpful for most genital infections

Serodiagnostic methods have little use in diagnosis of chlamydial genital infection because of the difficulty of distinguishing current from previous infection. Detection of IgM antibodies against *C. trachomatis* is helpful in cases of infant pneumonitis. Chlamydial serology is also useful in the diagnosis of LGV, where a single high complement fixation antibody titer ($>1:32$) or a fourfold rise supports a presumptive diagnosis. The most satisfactory method for diagnosis of LGV is isolation of an LGV strain of *C. trachomatis* from aspirated bubos or tissue biopsies. In 80 to 90% of patients, the LGV complement fixation test is positive (titer $> 1:64$) shortly after the appearance of the bubo.

## TREATMENT

Effective antimicrobics include erythromycin, azithromycin, and doxycycline

Strains of *C. trachomatis* are sensitive to tetracyclines, macrolides and related compounds, and some fluoroquinolones. Azithromycin is given as a single oral dose for non-LGV *C. trachomatis* infection. Erythromycin is used for pregnant women and infants because of the tooth staining that may result from tetracycline therapy and less experience with the newer agents. Doxycycline is an alternative for *C. trachomatis* and is the drug of choice for treating LGV.

Prevention of reinfection most important control measure

For trachoma, a single dose of azithromycin is now the treatment of choice, although a tetracycline for 14 days is an alternative. Corrective surgery may prevent blindness and is required for severe corneal and conjunctival scarring. Control of trachoma is directed toward prevention of continued reinfection during early childhood. Improvement in general hygienic practices is the most important factor in decreasing transmission of infection within families and, of course, one of the most difficult to implement on a broad scale.

## PREVENTION

Prophylaxis for infants using topical erythromycin or silver nitrate on the conjunctiva has limited effectiveness for *Chlamydia,* because 15 to 25% of exposed infants still develop inclusion conjunctivitis. The primary approach to prevention of all forms of genital and infant *C. trachomatis* infection comprises detection of this infection in sexually active individuals and appropriate treatment, including infected women late in pregnancy. No vaccine is available or under development.

Primary approach is detection and treatment of infection in high-risk individuals

No vaccine available

# *Chlamydia psittaci*

## EPIDEMIOLOGY

Human psittacosis (ornithosis) is a zoonotic pneumonia contracted through inhalation of respiratory secretions or dust from droppings of infected birds. It was initially described in psittacines, such as parrots and parakeets, but was subsequently shown to occur in a wide range of avian species, including turkeys. Human infections have also been linked to livestock and cat reservoirs. The disease is usually latent in its natural host but may become active, particularly with the stress of recent captivity or transport; *C. psittaci* is then excreted in large amounts.

Pneumonia contracted from birds

Psittacosis in humans is seen mainly as an occupational hazard of poultry workers and bird fanciers, particularly owners of psittacine birds. Reported cases of human psittacosis in the United States decreased during the 1950s, in association with the use of antimicrobials in poultry feeds and quarantine regulations for imported psittacine birds. Currently 100 to 200 cases are reported each year. Some strains of *C. psittaci* are highly contagious and pose a hazard for laboratory workers processing specimens for *C. psittaci* isolation.

Associated with poultry processing and captive psittacine birds

## CLINICAL DISEASE AND TREATMENT

Psittacosis in humans is an acute infection of the lower respiratory tract, usually presenting with acute onset of fever, headache, malaise, muscle aches, dry hacking cough, and bilateral interstitial pneumonia. Occasionally, systemic complications such as myocarditis, encephalitis, endocarditis, and hepatitis may develop. The liver and spleen are often enlarged. The diagnosis of psittacosis should be suspected in any patient with acute onset of febrile lower respiratory illness who gives a history of close exposure to birds. Indeed, a history of bird exposure should be especially sought in patients who appear to have a bilateral pneumonia not proven to be caused by other agents. It must be remembered that spread can occur from both symptomatic and asymptomatic infections of birds. The specific diagnosis is usually made by demonstrating seroconversion, or a fourfold rise in the titer of complement-fixing or indirect fluorescent antibody to chlamydial group antigen. Although *C. psittaci* can be isolated from blood or sputum early in the disease, these methods are attempted only in specialized laboratories because of the risk of laboratory infection. Treatment with tetracycline or erythromycin is effective if given early in the course of illness.

Interstitial pneumonia is bilateral

Diagnosis is primarily serologic

Treatment with tetracycline or erythromycin

# *Chlamydia pneumoniae*

*C. pneumoniae* has been shown to be a cause of "walking pneumonia" in adults worldwide. It is estimated that 10% of pneumonia and 5% of bronchitis cases are due to this agent. Epidemiologic evidence indicates that infection occurs throughout the year and is

Clinical manifestations are similar to *M. pneumoniae* spread between humans by person to person contact. Unlike psittacosis, birds are not the reservoir. Outbreaks of community-acquired pneumonia caused by *C. pneumoniae* have been reported, as has apparent nosocomial spread. Reinfections occur, and clinically evident *C. pneumoniae* infection may be more evident in the elderly than in younger individuals. Most infections present as pharyngitis, lower respiratory tract disease, or both, and the clinical spectrum is similar to that of *Mycoplasma pneumoniae* infection. Pharyngitis or laryngitis may occur 1 to 3 weeks prior to bronchitis or pneumonia, and cough may persist for weeks. The diagnosis is established by serologic testing or culture, but these tests are not routinely available. Treatment with tetracycline or erythromycin is effective in ameliorating the signs and symptoms of *C. pneumoniae* infection. Currently, there is ongoing scientific interest in the potential role of persistent infection by *C. pneumoniae* in the pathogenesis of human vascular endothelial and intimal diseases, such as atherosclerosis.

Possible role in atherosclerosis is proposed

Treatment is tetracycline or erythromycin

## ADDITIONAL READING

Buimer M, et al. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by ligase chain reaction–based assays with clinical specimens from various sites: Implications for diagnostic testing and screening. *J Clin Microbiol* 1996;34:2395–2400. A report of LCR and review of other tests for diagnosis.

Kuo CC, et al. *Chlamydia pneumoniae* (TWAR). *Clin Microbiol Rev* 1995;8:451–461. An excellent general review.

Schachter J. Biology of *Chlamydia trachomatis.* In Holmes KK et al (eds). *Sexually Transmitted Diseases,* 3rd ed. New York: McGraw Hill; 1999; pp. 391–405. A comprehensive review of the basic biology and pathogenesis of chlamydia.

Schnoles D, et al. Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. *N Engl J Med* 1997;334:1362–1366. Indicates the important role of *C. trachomatis* in producing pelvic inflammatory disease and infertility as well as the means to prevent these serious complications.

CHAPTER 31

# *Rickettsia, Coxiella, Ehrlichia,* and *Bartonella*

W. Lawrence Drew

The terminology of the rickettsiae and rickettsiae-like organisms has been revised very recently. Rickettsiae are the causes of spotted fevers and typhus and related illnesses, but the cause of scrub typhus is now *Orientia tsutsugamushi*. Ehrlichia is a family distinct from true rickettsiae and has two medically important genera: *Ehrlichia* and *Anaplasma*. *Coxiella burnetii,* the cause of Q fever, is a bacterium which is no longer classified with the rickettsiae. All are Gram-negative bacilli, and all are strict intracellular pathogens. The reservoir is animals, and, except for Q fever, all are transmitted by arthropod vectors. The diseases are typically fevers, often with vasculitis. The most common infections are the various spotted fevers found throughout the world.

Obligate intracellular parasites

*Bartonella* is a genus of Gram-negative bacilli formerly classified with the rickettsiae. However, this is not a group of strict intracellular pathogens.

## RICKETTSIA

## BACTERIOLOGY

### MORPHOLOGY AND STRUCTURE

Rickettsiae are small coccobacilli that often have a transverse septum between two bacilli, reflecting division by binary fission. They commonly measure no more than 0.3 to 0.5 $\mu$m. Although the Gram reaction is negative, they take the usual bacterial stains poorly and are better demonstrated by the Giemsa stain, particularly in infected cells. The ultrastructural morphology, which is similar to that of other Gram-negative bacteria, includes a Gram-negative type of cell envelope, ribosomes, and a nuclear body. Chemically, the cell wall contains lipopolysaccharide and at least two large proteins in the outer membrane, as well as peptidoglycan. The outer membrane proteins extend to the cell surface, where they are the most abundant protein present.

Small, Gram-negative coccobacilli stained best by Giemsa

Abundant outer membrane proteins at surface

## GROWTH AND METABOLISM

Grow in cytoplasm following induced endocytosis

Growth slow compared to most bacteria

Spread involves actin polymerization

*Rickettsia* grow freely in the cytoplasm of eukaryotic cells to which they are highly adapted, in contrast to *Ehrlichia* and *Coxiella,* which replicate in cytoplasmic vacuoles. Rickettsiae can be grown only in living host cells such as cell cultures and embryonated eggs. Infection of the host cell begins by induction of an endocytic process, which is analogous to phagocytosis, but requires expenditure of energy by the rickettsiae. Penetration of infected cells appears to be facilitated by production of a rickettsial phospholipase. The organisms then escape the phagosome or endocytic vacuole to enter the cytoplasm, possibly aided by elaboration of the phospholipase. Recent studies indicate that intracellular and intercellular spread involves directional actin polymerization and use of the host cell cytoskeleton in a manner similar to *Listeria* (see Chapter 18) and *Shigella* (see Chapter 21). Intracytoplasmic growth eventually produces lysis of the cell. The estimated generation time of rickettsiae is much longer than that of bacteria such as *Escherichia coli* but more rapid than that of *Mycobacterium tuberculosis.*

Exogenous cofactors and ATP required for survival

Rapidly loses infectivity outside of host cell

The obligate intracellular parasitism of rickettsiae has several interesting features. Failure to survive outside the cell is apparently related to requirements for nucleotide cofactors (coenzyme A, nicotinamide adenine dinucleotide) and adenosine triphosphate (ATP). Outside the host cell, rickettsiae not only cease metabolic activity, but leak protein, nucleic acids, and essential small molecules. This instability leads to rapid loss of infectivity, because the penetration of another cell requires energy. In summary, rickettsiae have the metabolic capabilities of other bacteria, but must borrow some essential elements from host cells for adequate growth and, thus, do not survive well in the environment.

# RICKETTSIAL DISEASE

CLINICAL CAPSULE

The classic example of rickettsial disease is epidemic typhus, but the most important rickettsiosis in the United States is Rocky Mountain spotted fever (RMSF). Both types of rickettsial disease are characterized by fever, rash, and myalgias/myositis. In RMSF, the rash appears first on the palms and soles, wrists, and ankles, and it migrates centripetally; in epidemic typhus, the rash begins on the trunk and spreads to the extremities, traveling in the opposite direction. Both diseases may be fatal as the result of severe vascular collapse. The vectors also differ; for RMSF, the vector is a tick, and for epidemic typhus, a louse.

## EPIDEMIOLOGY AND PATHOGENESIS

Infect vascular endothelium with resultant vasculitis and thrombosis

Multiple vascular lesions, including adrenal glands

Most rickettsiae have animal reservoirs and are spread by insect vectors, which are prominent components of their life cycles (Table 31–1). Most rickettsial infections of humans result in clinical illness. Rickettsiae infect the vascular endothelium, and the primary pathologic lesion is a vasculitis in which rickettsiae multiply in the endothelial cells lining the small blood vessels. Focal areas of endothelial proliferation and perivascular infiltration leading to thrombosis and leakage of red blood cells into the surrounding tissues account for the rash and petechial lesions. Vascular lesions occur throughout the body, producing the systemic manifestations of the disease. They are obviously most apparent in skin but most serious in the adrenal glands. An endotoxin-like shock has been demonstrated in animals on injection of whole rickettsial cells, but the nature and role of any toxin in human disease are unknown.

## DIAGNOSIS

In vitro cultivation is hazardous

Culture of rickettsiae is both difficult and hazardous. Their isolation in fertile eggs or cell cultures is generally attempted only in reference centers with special facilities and personnel experienced in handling the organisms. For this reason, serologic tests are the primary means of specific diagnosis. A number of test systems using specific rickettsial

TABLE 31–1

**Examples of Pathogenic Rickettsiae**

| Disease | Organism | Most Common Geographic Distribution | Zoonotic Cycle: Vector | Zoonotic Cycle: Reservoir |
|---|---|---|---|---|
| Spotted fever group Rocky Mountain spotted fever | *Rickettsia rickettsii* | North and South America | Tick | Rodents, dogs |
| Rickettsialpox | *Rickettsia akari* | United States former Soviet Union, Korea, Africa | Mite | Mouse |
| Mediterranean spotted fevers | *Rickettsia conorii* | Southern Mediterranean, Israel, Africa | Tick | Rodents, dogs |
| Typhus group epidemic | *Rickettsia prowazekii* | Africa, Asia, South America | Body louse | Humans[a] |
| Brill's | *Rickettsia prowazekii* | Worldwide[b] | None[c] | Humans |
| Murine | *Rickettsia typhi* | Worldwide (pockets) | Flea | Rodents |
| Scrub | *Orientia tsutsugamushi* | South Pacific, Asia | Mite | Rodents |
| Trench fever | *Bartonella quintana*[d] | Europe, Africa, Asia | Body louse | Humans |
| Q fever | *Coxiella burnetii* | Worldwide | None[e] | Sheep, cattle, goats |
| Cat scratch fever | *Bartonella henselae*[d] | Worldwide | None | Cats, dogs |
| Human ehrlichiosis | *Ehrlichia* (several species), *Anaplasma phagocytophilum* | Worldwide | Ticks | Dogs, deer, rodents |

[a] An apparently identical organism has been isolated from flying squirrels in the United States.
[b] Related to immigration.
[c] Relapsing form of epidemic typhus.
[d] Related to *Rickettsia* but has been grown in artificial culture.
[e] Transmission by inhalation of infected aerosols.

antigens have been developed, of which the indirect fluorescent antibody (IFA) method is generally the most sensitive and specific. This test is usually available only in reference laboratories. For rapid diagnosis, examination of biopsies such as skin lesions by immunofluorescence or immunoenzyme methods to detect antigens can be used.

IFA method usually employed for serologic diagnosis

# RICKETTSIAL DISEASE: CLINICAL ASPECTS

## SPOTTED FEVER GROUP

The most important rickettsial disease in North America is RMSF, which is caused by *Rickettsia rickettsii.* A number of other spotted fever rickettsioses are found in other parts of the world (see Table 31–1); the name often reveals the locale (eg, Mediterranean spotted fever, Marseilles fever). They are caused by *Rickettsia conorii,* a species serologically related to, but distinct from, *R. rickettsii.* Another less severe spotted fever, rickettsialpox, also occurs in North America.

Many tick-borne rickettsioses occur throughout the world

### Rocky Mountain Spotted Fever

RMSF is an acute febrile illness that occurs in association with residential and recreational exposure to wooded areas where infected ticks exist. The disease has a significant mortality (25%) if untreated.

## Epidemiology

Ticks naturally infected

Transovarial spread perpetuates tick infection

*R. rickettsii* is primarily a parasite of ticks. In the western United States, the wood tick (*Dermacentor andersoni*) is the primary vector. In the East, the dog tick (*Dermacentor variabilis*) is the natural carrier and vector of the disease, and in the Southwest and Midwest, the vector is the Lone Star tick (*Amblyomma americanum*). *R. rickettsii* does not kill its arthropod host, so the parasite is passed through unending generations of ticks by transovarial spread. Adult females require a blood meal to lay eggs and thus may transmit the disease. Infected adult ticks have been shown to survive as long as 4 years without feeding.

Most cases in children

*R. rickettsii* is found in both North America and South America. The highest attack rates in the United States are in the central and mid-Atlantic states (Fig 31–1). The US incidence increased in the 1970s and early 1980s to more than 0.5 cases per 100,000 population but has since decreased to less than half that figure. More than two thirds of cases are in children less than 15 years of age. The illness is generally seen between April and September because of increased exposure to ticks. A history of tick bite can be elicited in approximately 70% of cases.

## Manifestations

Incubation period 2–6 days after tick bite

The incubation period between the tick bite and the onset of illness is usually 2 to 6 days but may be as long as 2 weeks. Fever, headache, rash, toxicity, mental confusion, and myalgia are the major clinical features. The rash is the most characteristic feature of the illness. It usually develops on the second or third day of illness as small erythematous macules that rapidly become petechial. The lesions appear initially on the wrists and ankles and then spread up the extremities to the trunk in a few hours. A diagnostic feature of

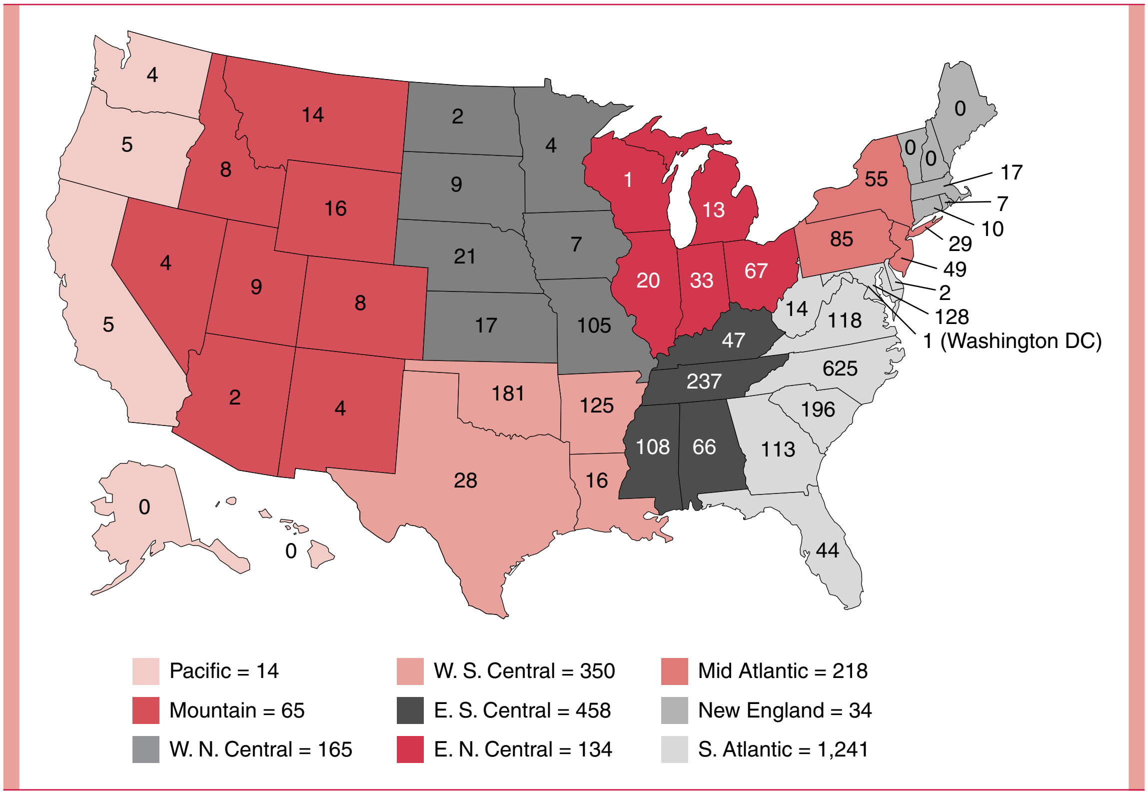


FIGURE 31–1

Rocky Mountain spotted fever. Number of cases in the United States in 1992. (*Reprinted with permission from Centers for Disease Control and Prevention. Summary of Notifiable Diseases, United States 1992.* MMWR Morb Mortal Wkly Rep *1993;41(55).*)

RMSF is the frequent appearance of the rash on the palms and soles, a finding not usually seen in maculopapular eruptions associated with viral infections. Muscle tenderness, especially in the gastrocnemius, is characteristic and may be extreme. If untreated, or in occasional cases despite therapy, complications such as disseminated intravascular coagulation, thrombocytopenia, encephalitis, vascular collapse, and renal and heart failure may ensue.

Rash spreads from extremities to trunk and often involves palms and soles

## *Diagnosis*

Because serologic testing is the primary diagnostic approach, it is often difficult to establish the diagnosis of RMSF early in the course of illness. However, antibodies may appear by the sixth or seventh day of illness, and a fourfold rise in antibody titer between acute serum and convalescent serum establishes the diagnosis. Specific therapy must usually be started solely on the basis of clinical signs, symptoms, and epidemiologic considerations.

Rising antibody titers confirm diagnosis

Prompt initiation of therapy based on clinical and epidemiologic considerations

## *Treatment*

Appropriate antibiotic therapy is highly effective if given during the first week of illness. If delayed into the second week or when pathologic processes such as disseminated intravascular coagulation are present, therapy is much less effective. The antibiotic of choice is doxycycline. Sulfonamides may worsen the disease process and are thus contraindicated. Before specific therapy became available, the mortality of RMSF was approximately 25%. Treatment has reduced this figure to between 5 and 7%. Death results primarily in patients in whom diagnosis and therapy are delayed into the second week of illness.

Treatment during first week most effective

Doxycycline is the treatment of choice

## *Prevention*

The major means of preventing RMSF is avoidance or reduction of tick contact. Frequent deticking in tick-infested areas is important, because ticks generally must feed for 6 hours or longer before they can transmit the disease. Tick surveys in the Carolinas have shown infection in about 5% of samples. Killed vaccines prepared from infected ticks, or rickettsias grown in embryonated eggs and cell cultures have been developed. None is licensed for clinical use at present.

Frequent deticking, avoidance, and protective clothing important in prevention

## Rickettsialpox

Rickettsialpox was first recognized in New York City in 1946. It is a benign rickettsial illness caused by *Rickettsia akari* and transmitted by a rodent mite. Distinguishing features of the disease include an eschar at the site of the bite and a vesicular rash. The house mouse and other semidomestic rodents are the primary reservoir. Humans acquire infection when the mite seeks an alternative host.

Benign disease transmitted by rodent mites

Rickettsialpox is a biphasic illness. The first phase is the local lesion at the bite, which starts as a papulovesicle and develops into a black eschar in 3 to 5 days. Fever and constitutional symptoms appear as the organism disseminates. The second phase of the disease is a diffuse rash similar to that of RMSF distributed randomly in the body, which, like the local lesion, becomes papulovesicular and develops into eschars. Rickettsialpox is self-limiting after 1 week, and no deaths have been reported. Tetracycline therapy shortens the course to 1 to 2 days.

Local eschar followed by fever and rash

Tetracycline therapy

# TYPHUS GROUP

## Epidemic Louse-Borne Typhus Fever

Primary louse-borne typhus fever is caused by *Rickettsia prowazekii,* which is transmitted to humans by the body louse. Historically, it has appeared during times of misery (war, famine) that create conditions favorable to human body lice (crowding, infrequent bathing). Although foci of endemic typhus are thought to persist in parts of Africa and Latin America, the number of reported cases has declined in recent decades. Most come

Severe louse-borne disease due to *R. prowazekii*

Endemic foci in Africa

from a single country, Ethiopia, which has had more than its share of social upheaval. Epidemic typhus has not been seen in the United States for more than half a century. *R. prowazekii* has been recovered from flying squirrels and their ectoparasites in the southeastern United States, and a few human cases of sylvatic typhus have occurred in these areas.

Infection involves feeding and defecation by louse

The chain of epidemic typhus infection starts with *R. prowazekii* circulating in a patient's blood during an acute febrile infection. The human body louse becomes infected during one of its frequent blood meals, and after 5 to 10 days of incubation, large numbers of rickettsiae appear in its feces. As the louse defecates while it feeds, the organisms can be rubbed into the louse bite wounds when the host scratches the site. Dried louse feces are also infectious through the mucous membranes of the eye or respiratory tract. The louse dies of its infection in 1 to 3 weeks, and the rickettsiae are not transmitted transovarially.

Fever, headache, and rash with high mortality rate

Louse control is primary prevention

Fever, headache, and rash begin 1 to 2 weeks after the bite. A maculopapular rash appears first on the trunk and then spreads centrifugally to the extremities, a pattern opposite to that of RMSF. Headache, malaise, and myalgia are prominent components of the illness. Complications include myocarditis and central nervous system dysfunction. In untreated disease, the fatality rate increases with age from 10% to as high as 60%. Therapy with tetracycline or chloramphenicol is effective. Louse control is the best means of prevention and is particularly important in controlling epidemics. No effective vaccine is available.

## *Recrudescent Typhus*

Less severe relapse of typhus after many years

Recrudescent typhus (**Brill's disease**) is a relapse of louse-borne typhus appearing 10 to 40 years after the primary attack. Factors triggering the relapse are unknown, but may involve fading immunity to rickettsiae that have remained dormant in reticuloendothelial cells. Recrudescent typhus is usually milder than the primary infection and is less often fatal, presumably because of partial immunity.

## *Endemic Typhus*

Transmitted by rat fleas

Endemic or murine typhus is caused by *Rickettsia typhi* and transmitted to humans by the rat flea (*Xenopsylla cheopis*). Human illness is incidental to the natural transmission of the disease among urban rodents, which serve as the reservoir. Only 30 to 60 cases of murine typhus are reported in the United States each year. Half of these typically occur along the Gulf Coast of Texas.

Resembles typhus but less severe

*R. typhi* shares antigens with *R. prowazekii*

The pathogenesis is similar to that of louse-borne typhus but the history includes exposure to rats, rat fleas, or both. The flea defecates when it takes a blood meal, and the infected feces gain access through the bite wound. After an incubation period of 1 to 2 weeks, illness begins with headache, myalgia, and fever. The rash is maculopapular, not petechial; it starts on the trunk and then spreads to the extremities in a manner similar to typhus. Because of antigens shared by *R. typhi* and *R. prowazekii,* serologic tests may not separate the two diseases. In the untreated patient, fever may last 12 to 14 days. With tetracycline or chloramphenicol therapy, the course is reduced to 2 to 3 days. Mortality and complications are rare, even if the disease is untreated.

## *Scrub Typhus*

Scrub typhus transmitted by rodent mite larvae (chiggers)

Scrub typhus is found in the southwest Pacific, Southeast Asia, and Japan. The causative organism is *Orientia tsutsugamushi,* a rickettsial organism. Mites that infest rodents are the reservoir and vectors, transmitting the rickettsiae to their own progeny via infected ova. Humans pick up the mites as they pass by low trees or brush. The mite larvae (chiggers) deposit rickettsiae as they feed.

Local eschar followed by fever, headache, rash, and lymphadenopathy

The typical initial lesion, a necrotic eschar at the site of the bite on the extremities, develops in only 50 to 80% of cases. Fever increases slowly over the first week, sometimes reaching 40.5°C. Headache, rash, and generalized lymphadenopathy follow later.

The maculopapular rash, which appears after about 5 days, is more evanescent than that seen with louse-borne or murine typhus. Hepatosplenomegaly and conjunctivitis may also appear. Specific diagnosis requires demonstration of a serologic response using the IFA test. The prognosis is good with chloramphenicol or tetracycline therapy, but the mortality of untreated patients is as high as 30%.

Serologic diagnosis by IFA

# *COXIELLA*

## BACTERIOLOGY

*Coxiella burnetii,* the cause of **Q fever,** has morphologic features similar to those of rickettsiae but differs in DNA composition and a number of other features. Phase variation of surface polysaccharide in response to environmental conditions has been observed and linked to virulence. The organism is taken into host cells by a phagocytic process that in contrast to rickettsiae does not involve expenditure of energy by the parasite. It multiplies in the phagolysosome primarily because it is adapted to growth at low pH and resists lysosomal enzymes. *C. burnetii* is much more resistant to drying and other environmental conditions than rickettsiae, which substantially accounts for its ability to produce infection by the respiratory route.

Multiplies in phagolysosome

Resistant to drying

## *COXIELLA* INFECTION: Q FEVER

Q fever is primarily a zoonosis transmitted from animals to humans by inhalation rather than by arthropod bite. Its distribution is worldwide among a wide range of mammals, of which cattle, sheep, and goats are most associated with transmission to humans. *C. burnetii* grows particularly well in placental tissue, attaining huge numbers ($>10^{10}$ per gram), which at the time of parturition contaminate the soil and fomites, where it may survive for years. Q fever occurs in those individuals exposed to infected animals or their products, particularly workers involved with slaughtering. Another high-risk environment is animal research facilities that have not provided adequate protection for personnel. Infection in all of these circumstances is believed to result from inhalation, which may be at some distance from the site of generation of the infectious aerosols. Infection can also occur from ingestion of animal products such as unpasteurized milk.

Transmission usually by inhalation; occasionally by ingestion

Occupational exposure in abattoirs and research facilities

## Q FEVER: CLINICAL ASPECTS

*C. burnetii* has an affinity for the reticuloendothelial system, but little is known of the pathology, because fatal cases are rare. As in livestock, most human infections are inapparent. When clinically evident, Q fever usually begins 9 to 20 days after inhalation, with abrupt onset of fever, chills, and headache. A mild, dry, hacking cough and patchy interstitial pneumonia may or may not be present. There is no rash. Hepatosplenomegaly and abnormal liver function tests are common. Complications such as myocarditis, pericarditis, and encephalitis are rare. Chronic infection is also rare but particularly important when it takes the form of endocarditis. There is evidence that the strains associated with endocarditis constitute an antigenic subgroup of *C. burnetii.*

Systemic infection without rash

Pneumonia and endocarditis may occur

Diagnosis is usually made by demonstrating high or rising titers of antibody to Q fever antigen by complement fixation, IFA, or enzyme immunoassay procedures. Although most infections resolve spontaneously, tetracycline therapy is believed to shorten the duration of fever and reduce the risk of chronic infection. Vaccines have been shown to stimulate antibodies, and some studies have suggested a protective effect for heavily exposed workers.

Diagnosis is serologic

FIGURE 31–2
Mononuclear cell in the cerebrospinal fluid containing *Ehrlichia* intracytoplasmic inclusions (arrow). (*Reprinted with permission from Dunn BE, Monson TP, Dumler JS, et al. Identification of* Ehrlichia chaffeensis *morulae in cerebrospinal fluid mononuclear cells.* J Clin Microbiol *1992;30:2207–2210.*)

## EHRLICHIA

The *Ehrlichia* genus includes several species of white blood cell (WBC)–associated bacteria that cause human disease. *Ehrlichia sennetsu,* the first species to be identified as a cause of human disease, is restricted to Japan and Malaysia. In the United States, two species are the principal causes of two diseases: (1) human monocytic ehrlichiosis (HME), which is due to *Ehrlichia chaffeensis;* and human granulocytic ehrlichiosis (HGE), which is due to *Anaplasma phagocytophilum. E. chaffeensis* infections tend to occur in the southeastern and lower midwestern United States, whereas the other infections tend to cluster in the northern states, with a distribution similar to Lyme disease (see Chapter 27). They were first reported to cause human disease in the 1950s. HGE is the predominant form of ehrlichiosis and is second only to Lyme disease as a tickborne infection in the United States. They are transmitted by deer or dog ticks. Both HME and HGE are clinically similar to RMSF, but rashes are less commonly seen. Still another species, *E. ewingii,* causes dog ehrlichiosis, which is occasionally contracted by humans.

Tickborne and WBC associated

On occasion, the diagnosis of ehrlichiosis may be suggested by observation of characteristic ehrlichial intracytoplasmic inclusions (morulae) in granulocytes (HGE) or mononuclear cells (HME) (Fig 31–2). Confirmation is usually made serologically by a fourfold or greater rise in IFA antibody or a titer greater than or equal to 1:64 to the specific antigen. These tests require the assistance of specialized laboratories. Another diagnostic test for detection of ehrlichia DNA is the polymerase chain reaction (PCR). Laboratory clues to human ehrlichiosis include a falling leukocyte count, thrombocytopenia, anemia, and impaired liver and renal function.

Intracytoplasmic inclusions (morulae) in monocytes or granulocytes

Doxycycline is the drug of choice for ehrlichiosis. The risk of infection can be reduced by avoiding wooded areas and tick bites.

Treatment is doxycycline

## BARTONELLA

*Bartonella* species differ from rickettsiae in that they can be cultured on artificial media. By 16s ribosomal comparison they are actually more closely related to *Brucella* than to rickettsiae. *Bartonella quintana,* the best known species of this genus, causes **trench fever,** which has a worldwide distribution. The name derives from its prominence in the trenches of World War I. This disease has a reservoir in humans, and its vector is the body louse. Most cases are mild or subclinical. When symptomatic, the patient has sudden onset of chills, headache, relapsing fever, and a maculopapular rash on the trunk and abdomen. Illness can last for 14 to 30 days and the disease is suggested by a history of louse

*B. quintana* causes trench fever; it is also associated with alcoholism contact. More recently, *B. quintana* bacteremia and endocarditis have been described in homeless alcoholic men in both France and the United States. The diagnosis can be made by culturing the organism on special agar medium or by demonstrating seroconversion.

*Bartonella bacilliformis,* a related organism, is the cause of acute Oroya fever and, in its chronic phase, verruga peruana. Infections with this agent are seen only in South America at intermediate altitudes, in keeping with the distribution of its sandfly vector.

Another species, *Bartonella henselae,* has been associated with a number of diseases, the most common of which is **cat scratch disease.** Cat scratch disease is a febrile lymphadenitis with systemic symptomatology that sometimes persists for weeks to months. Approximately 24,000 cases occur in the United States each year. The disease is thought to be transmitted by cat scratches or bites and perhaps by the bites of cat fleas. Manifestations may include skin rashes, conjunctivitis, encephalitis, and prolonged fever. Occasionally, retinitis, endocarditis, and granulomatous or suppurative hepatosplenic and osseous lesions have also been seen. *B. henselae* has been isolated directly from the blood of cats, although the latter do not appear ill. It can also be isolated from human blood, lymph nodes, and other materials using special media. Organisms can sometimes be directly demonstrated in infected tissues by using the Warthin–Starry silver impregnation stain. A serologic response to *B. henselae* antigens is the primary method of diagnosis. Azithromycin or erythromycin may reduce the duration of lymph node enlargement and symptoms.

Cat scratch disease is common in children

Persistent lymphadenitis is the usual finding

Bacillary angiomatosis, a proliferative disease of small blood vessels of the skin and viscera, seen in acquired immunodeficiency syndrome (AIDS) patients and other immunocompromised hosts, has been associated with *Bartonella* by molecular methods. The PCR (see Chapter 14) was used to amplify ribosomal RNA gene fragments directly from tissue samples. Sequence analysis of DNA transcribed from these fragments pointed to the *Bartonella* genus. Subsequently, both *B. henselae* and *B. quintana* have been isolated from AIDS patients with bacillary angiomatosis. Other conditions seen primarily in AIDS patients, such as peliosis hepatis and bacteremia with fever, have also been associated with *B. henselae. Bartonella* infections in AIDS and other immunosuppressed patients, as well as the bacteremia observed in alcoholic and homeless men, generally respond to prolonged courses of erythromycin. *Bartonella* endocarditis usually requires valve replacement as well.

AIDS and other immunocompromised states are associated with more severe, protracted infections

## ADDITIONAL READING

Bakken JS, Dumier JS. Human granulocytic ehrlichiosis. *Clin Infect Dis* 2000;31:554–560. Reviews all aspects of this disease.

Bass JW, Vincent JM, Person DA. The expanding spectrum of *Bartonella* infections: II. Cat scratch disease. *Pediatr Infect Dis J* 1997;16:163–179. A historical and clinical review of *Bartonella* infections.

Jacobs RF, Schutze GE. Ehrlichiosis in children. *J Pediatr* 1997;131:184–192. An excellent treatise on both human granulocytic and monocytic forms.

Kelly DJ, Richards AL, Temenak J, et al. The past and present threat of rickettsial diseases to military medicine and international public health. *Clin Infect Dis* 2002;34:S145–S169. The authors present both historical and current perspectives on rickettsial diseases that are timely and highly informative.

La Scola B, Raoult D. Laboratory diagnosis of rickettsioses: Current approaches to diagnosis of old and new rickettsial diseases. *J Clin Microbiol* 1997;35:2715–2727. For those interested in learning more details of rickettsial diagnosis, this is an excellent review.

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CHAPTER 32

# Plague and Other Bacterial Zoonotic Diseases

KENNETH J. RYAN

Many bacterial, rickettsial, and viral diseases are classified as zoonoses, because they are acquired by humans either directly or indirectly from animals. This chapter considers bacteria causing four zoonotic infections that are not discussed in other chapters. All four species, *Brucella, Yersinia pestis, Francisella tularensis,* and *Pasteurella multocida,* are Gram-negative bacilli that are primarily animal pathogens. The diseases they cause, brucellosis, plague, tularemia, and pasteurellosis, are now rare in humans and develop only after unique animal contact. The full range of zoonoses considered in this and other chapters is shown in Table 32–1.

## *BRUCELLA*

### BACTERIOLOGY

*Brucella* species are small, coccobacillary, Gram-negative rods that morphologically resemble *Haemophilus* and *Bordetella.* They are nonmotile, non–acid fast, and non–spore forming. The cells have a typical Gram-negative structure and the outer membrane contains proteins and two major antigenic variants (A and M) whose relative proportion varies with species and growth conditions. Although DNA homology studies indicate that there is only a single species, medical microbiologists prefer to believe that there are six species, each of which is primarily associated with its own mammalian hosts. Of these, *Brucella melitensis* (sheep, goats)*, Brucella abortus* (cattle), and *Brucella suis* (pigs) are the most important in human disease. Their growth is slow, requiring at least 2 to 3 days of aerobic incubation in enriched broth or on blood agar. All species produce catalase, oxidase, and urease, but do not ferment carbohydrates. They are differentiated by carbon dioxide requirements, hydrogen sulfide production, and susceptibility to dyes (thionin and basic fuchsin).

Coccobacilli resemble *Haemophilus*

Species are associated with different mammals

TABLE 32–1

## Some Important Bacterial and Rickettsial Zoonotic Infections

| Disease | Etiologic Agent | Usual Reservoir | Usual Mode of Transmission to Humans | Transmission Between Humans | Mode of Transmission Between Humans | Special Characteristics |
|---|---|---|---|---|---|---|
| Anthrax | *Bacillus anthracis* | Cattle, sheep, goats | Infected animals or products | No[a] | | Resistant spores |
| Bovine tuberculosis | *Mycobacterium bovis* | Cattle | Milk | No[a] | | |
| Brucellosis | *Brucella* spp. | Cattle, swine, goats | Milk, infected carcasses | No[a] | | |
| *Campylobacter* infection | *C. jejuni* | Wild mammals, cattle, sheep, pets | Contaminated food and water | Yes | Fecal–oral | |
| Leptospirosis | *Leptospira* spp. | Cattle, rodents | Water contaminated with urine | No[a] | | |
| Lyme disease | *Borrelia burgdorferi* | Deer, rodents | Ticks; transplacentally | No[a] | | Relapsing disease |
| Pasteurellosis | *Pasteurella multocida* | Animal oral cavities | Bites, scratches | No[a] | | |
| Plague | *Yersinia pestis* | Rodents | Fleas | Yes | Droplet (pneumonic) spread | Great epidemic potential |
| Other *Yersinia* infections | *Y. enterocolitica, Y. pseudotuberculosis* | Wild mammals, pigs, cattle, pets | Fecal–oral | Yes | Fecal–oral | |
| Relapsing fever | *Borrelia* spp. | Rodents, ticks | Ticks | Yes | Body louse[b] | Epidemic potential |
| Salmonellosis | *Salmonella* serotypes | Poultry, livestock | Contaminated food | Yes | Fecal contamination of food | |
| Rickettsial spotted fevers | *R. rickettsii*[c] | Rodents, ticks, mites | Ticks, mites | No[a] | | |
| Murine typhus | *Rickettsia typhi* | Rodents | Fleas | No[a] | | |
| Q fever | *Coxiella burnetii* | Cattle, sheep, goats | Contaminated dust and aerosols | No[a] | | |

[a] What never? No never. What *never*? Well, hardly ever! (W. S. Gilbert, "H.M.S. Pinafore").

[b] The relationship between tickborne relapsing fever and epidemic relapsing fever by the body louse remains uncertain.

[c] One of several etiologic agents.

# BRUCELLOSIS

CLINICAL CAPSULE

Brucellosis is a genitourinary infection of sheep, cattle, pigs, and other animals. Humans such as farmers, slaughterhouse workers, and veterinarians become infected directly by occupational contact or indirectly by consumption of contaminated animal products such as milk. In humans, brucellosis is a chronic illness characterized by fever, night sweats, and weight loss lasting weeks to months. Because the infection is localized in reticuloendothelial organs, there are few physical findings unless the liver or spleen become enlarged. When patients develop a cycling pattern of nocturnal fevers, the disease has been called undulant fever.

## EPIDEMIOLOGY

Brucellosis, a chronic infection that persists for life in animals, is an important cause of abortion, sterility, and decreased milk production in cattle, goats, and hogs. It is spread among animals by direct contact with infected tissues and ingestion of contaminated feed and causes chronic infection of the mammary glands, uterus, placenta, seminal vesicles, and epididymis. Although the associations are not absolute, each species is linked to a different animal: *B. abortus* tends to infect cattle; *B. melitensis,* sheep and goats; and *B. suis,* pigs.

Causes abortion in cattle, goats, pigs

Humans acquire brucellosis by occupational exposure or consumption of unpasteurized dairy products. The bacteria may gain access through cuts in the skin, contact with mucous membranes, inhalation, or ingestion. In the United States, the number of cases has dropped steadily from a maximum of more than 6000 per year in the 1940s to the current level of less than 100 per year. Of these cases, 50 to 60% are in abattoir employees, government meat inspectors, veterinarians, and others who handle livestock or meat products. Consumption of unpasteurized dairy products, which accounts for 8 to 10% of infections, is the leading source in persons who have no connection with the meat processing or livestock industries. Some recent cases of this type have been associated with "health" foods. In the United States, the distribution of human cases of brucellosis includes virtually every state, but is concentrated in those with large livestock industries or proximity to Mexico (California, Texas). An outbreak of *B. melitensis* in Texas was traced to unpasteurized goat cheese brought in from Mexico.

Occupational disease for veterinarians

Unpasteurized dairy products and "health" foods are a risk

## PATHOGENESIS

All *Brucella* species are facultative intracellular parasites of epithelial cells and professional phagocytes. After they penetrate the skin or mucous membranes, they enter and multiply in macrophages in the liver sinusoids, spleen, bone marrow, and other components of the reticuloendothelial system. Understanding the mechanisms for intracellular survival is incomplete but involves suppression of the myeloperoxidase system, inhibition of phagosome–lysosome fusion, and impairment of monocyte cytokine production. Thus, intracellular events in monocytes determine the outcome of a *Brucella* infection. In cows, sheep, pigs, and goats, erythritol, a four-carbon alcohol present in chorionic tissue, markedly stimulates growth of *Brucella.* This stimulation probably accounts for the tendency of the organism to locate in these sites. The human placenta does not contain erythritol.

Facultative intracellular pathogen multiplies in macrophages

Erythritol in animal placentas stimulates growth

If not controlled locally, infection progresses with the formation of small granulomas in the reticuloendothelial sites of bacterial multiplication and with release of bacteria back into the systemic circulation. These bacteremic episodes are largely responsible for the recurrent chills and fever of the clinical illness. These events resemble the pathogenesis of typhoid fever (see Chapter 21).

## IMMUNITY

Although antibodies are formed in the course of brucellosis, there is little evidence they are protective. Control of disease is due to T cell–mediated cellular immune responses.

Immunity is T-cell mediated

Development of helper T cell–type responses and the production of cytokines (tumor necrosis factor-$\alpha$, tumor necrosis factor-$\beta$, interleukin-1, interleukin-2) are associated with the elimination of *Brucella* from macrophages.

## BRUCELLOSIS: CLINICAL ASPECTS

### MANIFESTATIONS

Recurrent bacteremia comes from reticuloendothelial sites

Night sweats and periodic fever continue without obvious organ focus

Brucellosis starts with malaise, chills, and fever 7 to 21 days after infection. Drenching sweats in the late afternoon or evening are common, as are temperatures in the range of 39.4 to 40°C. The pattern of periodic nocturnal fever (undulant fever) typically continues for weeks, months, or even 1 to 2 years. Patients become chronically ill with associated body aches, headache, and anorexia. Weight loss of up to 20 kg may occur during prolonged illness. Despite these dramatic effects, physical findings and localizing signs are few. Less than 25% of patients show detectable enlargement of the reticuloendothelial organs, the primary site of infection. Of such findings, splenomegaly is most common, followed by lymphadenopathy and hepatomegaly. Occasionally, localized infection develops in the lung, bone, brain, heart, or genitourinary system. These cases usually lack the pronounced systemic symptoms of the typical illness.

### DIAGNOSIS

Blood culture is primary method

Serologic tests may be useful

Definitive diagnosis requires isolation of *Brucella* from the blood or from biopsy specimens of the liver, bone marrow, or lymph nodes. Supplementation with carbon dioxide is needed for growth of *B. abortus.* The slow growth of some strains requires prolonged incubation of culture medium to achieve isolation. Blood cultures may require 2 to 4 weeks for growth, although most are positive in 2 to 5 days. The diagnosis is often made serologically but is subject to the same interpretive constraints as are all serologic tests. Antibodies that agglutinate suspensions of heat-killed organisms typically reach titers of 1:640 or more in acute disease. Lower titers may reflect previous disease or cross-reacting antibodies. Titers return to the normal range within a year of successful therapy.

### TREATMENT AND PREVENTION

Tetracyclines are effective

Pasteurization is primary prevention

Tetracyclines are the primary antimicrobics for the treatment of brucellosis. Doxycycline is preferred because of its pharmacologic characteristics. In seriously ill patients, streptomycin, gentamicin, or rifampin may be added. Although $\beta$-lactams are active in vitro, clinical response is poor, probably due to failure to reach the intracellular location of the bacteria. The therapeutic response is not rapid; 2 to 7 days may pass before patients become afebrile. Up to 10% of patients have relapses in the first 3 months after therapy.

Prevention is primarily by measures that minimize occupational exposure and by the pasteurization of dairy products. Control of brucellosis in animals involves a combination of immunization with an attenuated strain of *B. abortus* and eradication of infected stock. No human vaccine is in use.

# *YERSINIA PESTIS*

## BACTERIOLOGY

Member of Enterobacteriaceae

*Y. pestis* is a nonmotile, non–spore-forming, Gram-negative bacillus with a tendency toward pleomorphism and bipolar staining. It is a member of the Enterobacteriaceae and is discussed in Chapter 21 with other members of the genus *Yersinia.* It shares features of the other *Yersinia* pathogenic for humans (*Y. pseudotuberculosis, Y. enterocolitica*), such as virulence plasmids and *Yersinia* outer membrane proteins (Yops). In addition, *Y. pestis* has two additional virulence plasmids, which code for a protein capsular antigen called F1 and enzymes with phospholipase, protease, and fibrinolytic activity. *Y. pestis* also has its own adhesin similar to the invasins of the other *Yersinia.*

Yops, protein capsule, and enzymes are present

# PLAGUE

CLINICAL CAPSULE

Plague, an infection of rodents transmitted to humans by the bite of infected fleas, is the most explosively virulent disease known. Most cases begin with a painful swollen lymph node (bubo) from which the bacteria rapidly spread to the bloodstream. Plague pneumonia (Black Death) is produced by seeding from the bloodstream or from another patient with pneumonia. All forms cause a toxic picture with shock and death within a few days. No other disease regularly kills previously healthy persons so rapidly.

## EPIDEMIOLOGY

The term **plague** is often used generically to describe any explosive pandemic disease with high mortality. Medically, it refers only to infection caused by *Y. pestis,* and this application was justly earned, because *Y. pestis* was the cause of the most virulent epidemic plague of recorded human history, the Black Death of the Middle Ages. In the 14th century, the estimated population of Europe was 105 million; between 1346 and 1350, 25 million died of plague. Pandemics continued through the end of the 19th century and the early 20th century despite elaborate quarantine measures developed in response to the obvious communicability of the disease. Yersin isolated the etiologic agent in China in 1894 and named it after his mentor, Pasteur (*Pasteurella pestis*). The name was later changed to honor Yersin (*Yersinia pestis*).

Black Death continued into 20th century

Plague is a disease of rodents transmitted by the bite of rat fleas (*Xenopsylla cheopis)* that colonize them. It exists in two interrelated epidemiologic cycles, the **sylvatic** and the **urban** (Fig 32–1). Endemic transmission among wild rodents in the sylvatic (L. *sylvaticus,* belonging to or found in the woods) is the primary reservoir of plague. When infected rodents enter a city, circumstances for the urban cycle are created. Humans can enter the cycle from the bite of the flea in either environment. However, chances are greater in the urban setting.

Sylvatic transmission among rodents is primary reservoir

The plagues of the Middle Ages are examples of the urban cycle involving rats and humans. When food is scarce in the countryside, rats migrate to cities, which facilitates rat-to-rat transmission and brings the primary reservoir into closer contact with humans. When the number of nonimmune rats is sufficient, epizootic plague develops among them, with bacteremia and high mortality. Fleas feeding on the rats become infected, and the bacteria multiply in the intestinal tract of the fleas to numbers that eventually block the proventriculus. As an infected rat dies, its hungry fleas seek a new host, which is usually another rat but may be a human. The infected flea regurgitates *Y. pestis* from the proventriculus into the new bite wound. Therefore, the probability of transmission to humans is greatest when both rat population and rat mortality are high.

Rat migration to cities increases human risk

Fleas regurgitate into bite wounds

The bite of the flea is the first event in the development of a case of **bubonic plague,** which, even if serious enough to kill the patient, is not normally contagious to other humans. However, some patients with bubonic plague develop a secondary pneumonia by bacteremic spread to the lungs. This **pneumonic plague** is highly contagious person-to-person by the respiratory droplet route. It is not difficult to understand how rapid spread proceeds in conjunction with crowded unsanitary conditions and continued flea-to-human transmission. An urban plague epidemic is vividly described through the eyes of a physician in Albert Camus' novel *The Plague.*

Bubo is initial lesion

Bacteremia pneumonia is contagious

Although urban plague epidemics have been essentially eliminated by rat control and other public health measures, sylvatic transmission cycles persist in many parts of the world, including North America. These cycles involve nonurban mammals such as prairie

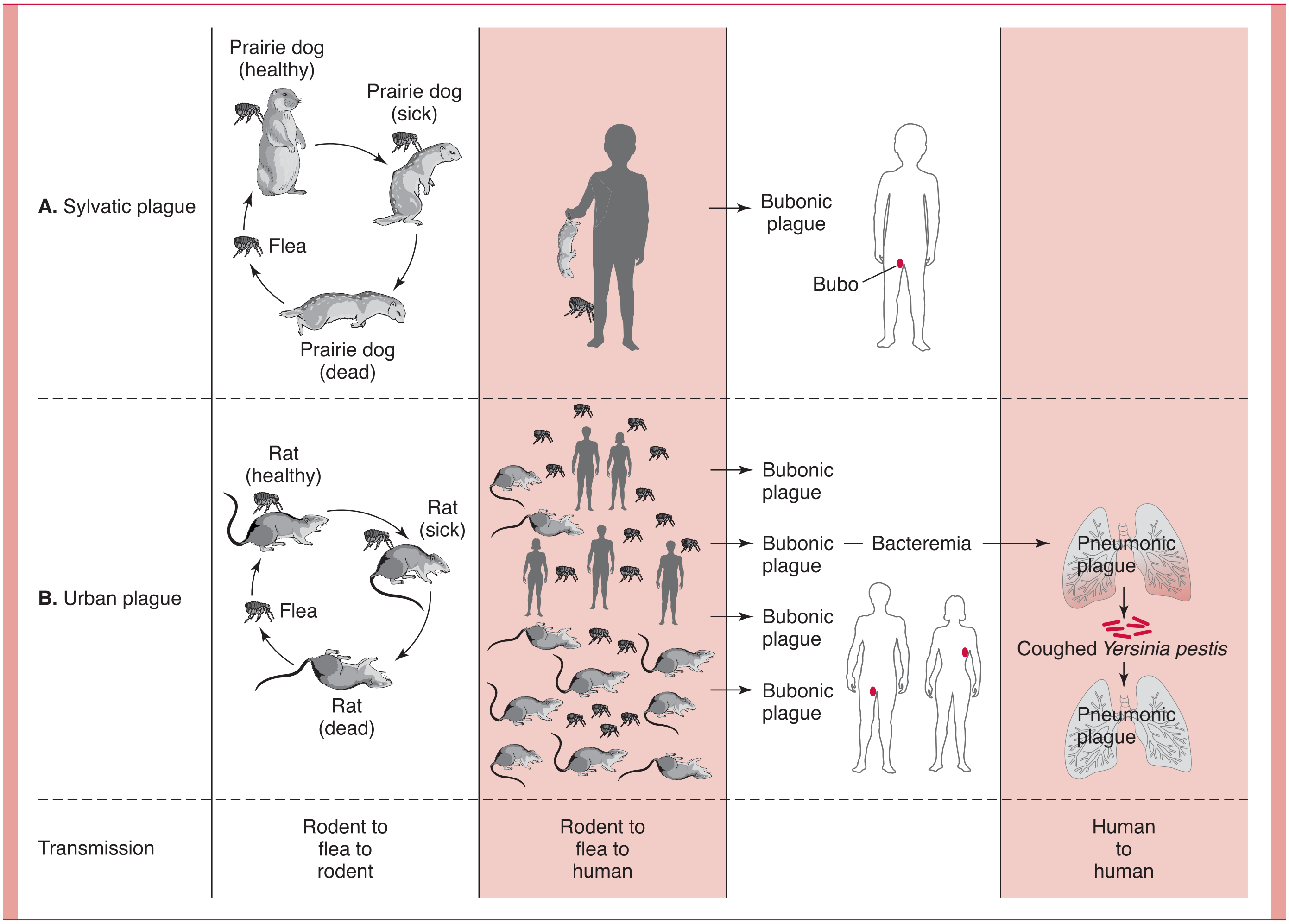


FIGURE 32–1

The epidemiology of plague. **A.** In the sylvatic cycle fleas leaving infected rodents, such as mice and prairie dogs, pass the infection to others in the population. Humans rarely contact these rodents but when they do, the flea bite transmits plague. **B.** In the urban cycle, masses of rats are in closer contact with humans and bites from infected fleas transmit the infection to many. In both cycles initial transmissions result in bubonic plague. Bacteremia with *Yersinia pestis* may infect the lungs to cause pneumonic plague. Pneumonic plague is transmitted human-to-human by the respiratory route without the involvement of fleas.

Nonepidemic disease is linked to animal contact

dogs, deer mice, rabbits, and wood rats. Transmission between them involves fleas. Coyotes or wolves may be infected by the same fleas or by ingestion of infected rodents. By their nature, the reservoir animals rarely come in contact with humans; when they do, however, the infected fleas they carry can transmit *Y. pestis.* The most common circumstance is a child exploring the outdoors who comes across a dead or dying prairie dog and pokes, carries, or touches it long enough to be bitten by the fleas leaving the animal. The result is a sporadic case of bubonic plague, which occasionally becomes pneumonic.

Most US cases are in western states

Pneumonia can be acquired from animals

Sylvatic plague, which exists in most continents, is common in Southeast Asia but is not found in Western Europe or Australia. In the United States, the primary enzootic areas are the semiarid plains of the western states. Infected animals and fleas have been detected from the Mexican border to the eastern half of Washington State. The geographic focus of human plague in the United States is in the "four corners" area where Arizona, New Mexico, Colorado, and Utah meet, but cases have occurred in California, west Texas, Idaho, and Montana. Most years, as many as 15 cases are reported, although this number rose to 30 to 40 in the mid-1980s. These variations are strongly related to changes in the size of the sylvatic reservoir. A fatal case of pneumonic plague reported in

1992 was linked to an infected domestic cat the patient had removed from the crawl space under a rural cabin in the endemic area.

## PATHOGENESIS

The plague cycle begins when a rat flea feeds on a rodent infected with *Y. pestis.* Bacteria are taken with the blood meal and multiply in the infected flea. Some virulence factors such as the fibrinolysin and phospholipase are produced at the ambient temperature (20–28°C), where they may enhance the multiplication of *Y. pestis* in the flea and facilitate the agglutination that blocks the flea gut proventriculus. The flea, sensing starvation, feeds voraciously and eventually regurgitates blood and bacteria into the bite wound. If this wound is in a new uninfected host (rat or human), a new case is created.

Multiplication in flea foregut is aided by low temperature virulence factors

Once injected past the skin barrier by the flea, *Y. pestis* produces a new set of virulence factors as it senses the change from the temperature and ionic environment of the flea to that of the new host. These include the Yops and an array of other virulence factors discussed in Chapter 21, plus the F1 capsular protein and a plasminogen-activating protease. The F1 protein forms a gel-like capsule, which has antiphagocytic properties that allow the bacteria to persist and multiply in the submucosa. The organisms eventually reach the regional lymph nodes through the lymphatics, where they multiply rapidly and produce a hemorrhagic suppurative lymphadenitis known clinically as the **bubo.** Spread to the bloodstream quickly follows. The extreme systemic toxicity that develops with bacteremia appears to be due to lipopolysaccharide (LPS) endotoxin combined with the many actions of Yops, proteases, and other extracellular products. The bacteremia causes seeding of other organs, most notably the lungs, producing a necrotizing hemorrhagic pneumonia known as pneumonic plague.

New virulence factors are triggered by temperature and ionic shift

Capsular protein is antiphagocytic

Bubo progresses to bacteremia

LPS and other products produce shock

## IMMUNITY

Recovery from bubonic plague appears to confer lasting immunity, but for obvious reasons the mechanisms in humans have not been extensively studied by modern immunologic methods. Animal studies suggest that antibody against the F1 capsular protein is protective by enhancing phagocytosis, but cell-mediated mechanisms are required for intracellular killing.

Anticapsular antibody may be protective

# PLAGUE: CLINICAL ASPECTS

## MANIFESTATIONS

The incubation period for bubonic plague is 2 to 7 days after the flea bite. Onset is marked by fever and the painful bubo, usually in the groin (**bubo** is from the Greek **boubon** for "groin") or, less often, in the axilla. Without treatment, 50 to 75% of patients progress to bacteremia and die in Gram-negative septic shock within hours or days of development of the bubo. About 5% of victims develop pneumonic plague with mucoid, then bloody sputum. Primary pneumonic plague has a shorter incubation period (2 to 3 days) and begins with only fever, malaise, and a feeling of tightness in the chest. Cough, production of sputum, dyspnea, and cyanosis develop later in the course. Death on the second or third day of illness is common, and there are no survivors without specific therapy. A terminal cyanosis seen with pneumonic plague is responsible for the term Black Death. Even today, plague pneumonia is almost always fatal if appropriate treatment is delayed more than a day from the onset.

Bubonic plague mortality is 50–75% in untreated cases

Pneumonic plague is fatal if untreated

Terminal cyanosis is the Black Death

## DIAGNOSIS

Gram smears of aspirates from the bubo typically reveal bipolar-staining Gram-negative bacilli. An immunofluorescence technique is available in public health laboratories for

Immunofluorescent staining is rapid

Cultures grow on routine media

immediate identification of smears or cultures. *Y. pestis* is readily isolated on the media used for other members of the Enterobacteriaceae (blood agar, MacConkey agar), although growth may require more than 24 hours of incubation. The appropriate specimens are bubo aspirate, blood, and sputum. Laboratories must be notified of the suspicion of plague to avoid delay in the bacteriologic diagnosis and to guard against laboratory infection.

## TREATMENT

Streptomycin is primary treatment

Streptomycin is the treatment of choice for both bubonic and pneumonic plague, because its effectiveness has been proven. Tetracycline, chloramphenicol, and trimethoprim–sulfamethoxazole are alternatives. Timely treatment reduces the mortality of bubonic plague below 10%. Of the 31 human cases of plague reported in the United States in 1984, 6 (19%) died.

## PREVENTION

Avoid sick or dead wild rodents

Tetracycline chemoprophylaxis is used for respiratory exposure

Urban plague has been prevented by rat control and general public health measures such as use of insecticides. Sylvatic plague is virtually impossible to eliminate because of the size and dispersion of the multiple rodent reservoirs. Disease can be prevented by avoidance of sick or dead rodents and rabbits. Eradication of fleas on domestic pets, which have been known to transport infected fleas from wild rodents to humans, is recommended in endemic areas. The continued presence of fully virulent plague in its sylvatic cycle poses a risk of extension to the urban cycle and epidemic disease in the event of major disaster or social breakdown. Chemoprophylaxis with tetracycline is recommended for those who have had close contact with a case of pneumonic plague. It is also used for the household contacts of a case of bubonic plague, because they may have had the same flea contact. A formalin-killed plague vaccine once used for those in high-risk occupations is no longer available.

# *FRANCISELLA*

## BACTERIOLOGY

Gram-negative coccobacilli have requirement for –SH compounds

*Francisella tularensis* is a small, facultative, coccobacillary, Gram-negative rod with much the same morphology as *Brucella*. It is one of the few bacterial species of medical importance that does not grow on the usual enriched media. This characteristic is due to a special requirement for sulfhydryl compounds, and growth occurs best on a cysteine–glucose blood agar medium incubated aerobically. On primary isolation, 2 to 10 days of incubation is required for appearance of the tiny transparent colonies. The species is antigenically homogeneous.

## TULAREMIA

**CLINICAL CAPSULE**

Tularemia is a disease of wild mammals caused by *F. tularensis*. Humans become infected by direct contact with infected animals or through the bite of a vector (tick or deer fly). The illness is characterized by high fever and severe constitutional symptoms. The epidemiology of tularemia and many features of the clinical infection are similar to those of plague.

## EPIDEMIOLOGY

Humans most often acquire *F. tularensis* by contact with an infected mammal or a bloodfeeding arthropod. Because the infecting dose is very low (<100 organisms), many routes of infection are possible. A tick bite or direct contact with minor skin abrasion are the most common mechanisms of infection. Many wild mammals can be infected, including squirrels, muskrats, beavers, and deer. A common history is that of skinning wild rabbits on a hunting trip. Inhalation may also lead to disease. In a recent outbreak of pulmonary tularemia on Cape Cod, experts believed that lawn mowing and brush cutting facilitated inhalation. Occasionally, the bite or scratch of a domestic dog or cat has been implicated when the animal has ingested or mouthed an infected wild mammal. Infected animals may not show signs of infection, because the organism is well adapted to its natural host. The usual vectors in animals are ticks and deer flies. Ticks may also serve as a reservoir of the organism by transovarial transmission to their offspring.

Infecting dose is low

Acquired by tick bites or directly from wild mammal

Tularemia is distributed throughout the Northern Hemisphere, although there are wide variations in specific regions. The highly virulent tick/rabbit-associated strains are common only in North America. In the United States 100 to 200 cases are reported each year half of which are in the lower Midwestern states (Arkansas, Missouri, Oklahoma). Tularemia is not found in the British Isles, Africa, South America, or Australia.

Distribution throughout Northern Hemisphere

## PATHOGENESIS

Relatively little is known of the events that occur during the 2- to 5-day incubation period. A lesion often develops at the site of infection, which becomes ulcerated. The organism then infects the reticuloendothelial organs, often forming granulomas, and the disease may sometimes follow a chronic relapsing course. These properties suggest a facultative intracellular pathogen; multiplication within macrophages, hepatocytes, and endothelial cells has been demonstrated with *F. tularensis.* This intracellular survival has been attributed to failure of phagosome–lysosome fusion and phagosome acidification. Early bacteremic spread probably occurs, although it is rarely detected. Other areas of multiplication are characterized by necrosis or granuloma production, and a mixture of abscesses and caseating granulomas may be seen in the same organ.

Intracellular survival in macrophages by phagosome control

## IMMUNITY

Naturally acquired infection appears to confer long-lasting immunity. Antibody titers remain elevated for many years, but cellular immunity plays the major role in resistance to reinfection. T cell–dependent reactions involving either CD4+ or CD8+ cell are detectable even before antibody responses.

Cell-mediated immunity is dominant

# TULAREMIA: CLINICAL ASPECTS

## MANIFESTATIONS

After an incubation period of 2 to 5 days, tularemia may follow a number of courses, depending on the site of inoculation and extent of spread. All begin with the acute onset of fever, chills, and malaise. In the ulceroglandular form, a local papule at the inoculation site becomes necrotic and ulcerative. Regional lymph nodes become swollen and painful. The oculoglandular form, which follows conjunctival inoculation, is similar except that the local lesion is a painful purulent conjunctivitis. Ingestion of large numbers of *F. tularensis* ($>10^8$) leads to typhoidal tularemia, with abdominal manifestations and a prolonged febrile course similar to that of typhoid fever. Inhalation of the organisms can result in pneumonic tularemia or a more generalized infection similar to the typhoidal form. Like plague pneumonia, tularemic pneumonia may also develop through seeding of the lungs by bacteremic

Ulceroglandular, oculoglandular, typhoidal, and pneumonic forms exist spread of one of the other forms. Any form of tularemia may progress to a systemic infection with lesions in multiple organs.

Ulceroglandular has lowest mortality

Without treatment, mortality ranges from 5 to 30%, depending on the type of infection. Ulceroglandular tularemia, the most common form, generally carries the lowest risk of a fatal outcome. In the US surveillance study mentioned earlier, the mortality was 2%.

## DIAGNOSIS

Special media are needed for culture

Serodiagnosis is most common

Because tularemia is uncommon and *F. tularensis* has unique growth requirements, the diagnosis is easily overlooked. Although some strains grow on chocolate agar, laboratories must be alerted to the suspicion of tularemia so that specialized media can be prepared and precautions taken against the considerable risk of laboratory infection. An immunofluorescent reagent is available in reference laboratories for use directly on smears from clinical material. Because of the difficulty and risk of cultural techniques, many cases are diagnosed by serologic tests. Agglutinating antibodies are usually present in titers of 1:40 by the second week of illness, increasing to 1:320 or greater after 3 to 4 weeks. Unless previous exposure is known, single high antibody titers are considered diagnostic.

## TREATMENT AND PREVENTION

Aminoglycosides are effective

Streptomycin is the drug of choice in all forms of tularemia, although recent experience indicates that gentamicin may be just as effective. Tetracycline and chloramphenicol have also been effective, but relapses are more common than with streptomycin. Prevention mainly involves the use of rubber gloves and eye protection when handling potentially infected wild mammals. Prompt removal of ticks is also important. A live attenuated vaccine exists, but it is used only in laboratory workers and those individuals who cannot avoid contact with infected animals.

# *PASTEURELLA MULTOCIDA*

Penicillin-sensitive, Gram-negative rods

Most common cause of infected animal bites or scratches

*P. multocida,* one of many species of *Pasteurella* in the respiratory flora of animals, is a cause of respiratory infection in some individuals. This small, coccobacillary, Gram-negative organism grows readily on blood agar but not on MacConkey agar. It is oxidase positive and ferments a variety of carbohydrates. Unlike most Gram-negative rods, *P. multocida* is susceptible to penicillin. Humans are usually infected by the bite or scratch of a domestic dog or cat. Infection develops at the site of the lesion, often within 24 hours. The typical infection is a diffuse cellulitis with a well-defined erythematous border. The diagnosis is made by culture of an aspirate of pus expressed from the lesion. Frequently, too few organisms are present to be seen on a direct Gram smear. *P. multocida* is by far the most common cause of an infected dog or cat bite. For unknown reasons, *P. multocida* is occasionally isolated from the sputum of patients with bronchiectasis. Infections are treated with penicillin.

## ADDITIONAL READING

Butler T. *Yersinia* infections: Centennial of the discovery of the plague bacillus. *Clin Infect Dis* 1994;19:655–663. A very readable account of Yersin's life, his discoveries, and features of *Yersinia* infections.

Crook LD, Tempest B. Plague. A clinical review of 27 cases. *Arch Intern Med* 1992;152:1253–1256. A nice review of clinical aspects of plague cases seen between

1965 and 1989 at the Gallup, New Mexico, Indian Medical Center. Analysis of treatment outcomes is included.

LeVier K, Phillips RW, Grippe VK, Roop II RM, Walker GC. Similar requirements of a plant symbiont and a mammalian pathogen for prolonged intracellular survival. *Science* 2000;287:2492–2493. This article presents a fascinating bit of interdisciplinary scientific detective work in which *Rhizobium meliloti,* a plant pathogen, is found to use the same mechanisms for intracellular survival as *Brucella abortus.*

McNeill WH. *Plagues and Peoples.* New York: Anchor Press/Doubleday; 1976. An account of the impact of infectious diseases, including zoonoses, on the course of human history.

Perry RD, Fetherston JD. *Yersinia pestis*—etiologic agent of plague. *Clin Microbiol Rev* 1997;10:35–66. A comprehensive review of bacteriology, epidemiology, and pathogenesis.

Taylor JP, Istre GR, McChesney TC, Satalowich FT, Parker RL, McFarland LM. Epidemiologic characteristics of human tularemia in the southwest-central states, 1981–1987. *Am J Epidemiol* 1991;133:1032–1038. This study indicates tularemia is more common in the United States than most experts thought.

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# PART VI

# PATHOGENIC VIRUSES

## CHAPTER 37

Hepatitis Viruses

## CHAPTER 38

Herpesviruses

## CHAPTER 39

Viruses of Diarrhea

## CHAPTER 40

Arthropod-Borne and Other Zoonotic Viruses

## CHAPTER 41

Rabies

## CHAPTER 42

Retroviruses, Human Immunodeficiency Virus, and Acquired Immunodeficiency Syndrome

## CHAPTER 43

Papovaviruses

## CHAPTER 44

Persistent Viral Infections of the Central Nervous System

# CHAPTER 33

# Influenza, Respiratory Syncytial Virus, Adenovirus, and Other Respiratory Viruses

C. GEORGE RAY

Respiratory disease accounts for an estimated 75 to 80% of all acute morbidity in the US population. Most of these illnesses (approximately 80%) are viral. If episodes not requiring medical attention are included, the overall average is three to four illnesses per year per person, although incidence varies inversely with age (the frequency is greater among young children). Seasonality is also a feature; incidence is lowest in the summer months and highest in the winter.

The viruses that are major causes of acute respiratory disease (ARD) include influenza viruses, parainfluenza viruses, rhinoviruses, adenoviruses, respiratory syncytial virus (RSV), and respiratory coronaviruses. Reoviruses are of questionable importance but are also considered. Others, such as enterovirus and measles virus, can also cause respiratory symptoms but are discussed in other chapters.

Respiratory viruses represented by diverse agents

In addition to the ability to cause a variety of ARD syndromes, this somewhat heterogeneous group of viruses shares a relatively short incubation period (1–4 days) and a person-to-person mode of spread. Transmission is direct, by infective droplet nuclei, or indirect, by hand transfer of contaminated secretions to nasal or conjunctival epithelium. All of these agents are associated with an increased risk of bacterial superinfection of the damaged tissue of the respiratory tract, and all have a worldwide distribution.

Short incubation period

Transmission by droplet nuclei or hands

## INFLUENZA VIRUSES

### INFLUENZA VIRUS GROUP CHARACTERISTICS

Influenza viruses are members of the **orthomyxovirus** group, which are enveloped, pleomorphic, single-stranded RNA viruses. They are classified into three major serotypes, A, B, and C, based on different ribonucleoprotein antigens. Influenza A viruses are the

TABLE 33–1

## Differences Among Influenza Viruses

| FEATURE | INFLUENZA A | INFLUENZA B | INFLUENZA C |
|---|---|---|---|
| Gene segments | 8 | 8 | 7 |
| Unique proteins | M2 | NB | HEF |
| Host range | Humans, swine, avians, equines, marine mammals | Humans only | Humans, swine |
| Disease severity | Often severe | Occasionally severe | Usually mild |
| Epidemic potential | Extensive; epidemics and pandemics (antigenic drift and shift) | Outbreaks; occasional epidemics (antigenic drift only) | Limited outbreaks (antigenic drift only) |

Orthomyxoviruses divided into types A, B, and C

Type A has greatest virulence and epidemic spread

most extensively studied of the three, and much of the following discussion is based on knowledge of this type. They generally cause more severe disease and more extensive epidemics than the other types; naturally infect a wide variety of species, including mammals and birds; and have a great tendency to undergo significant antigenic changes (Table 33–1). Influenza B viruses are more antigenically stable; are only known to naturally infect humans; and usually occur in more localized outbreaks. Influenza C viruses appear to be relatively minor causes of disease, affecting humans and pigs.

Enveloped RNA virus with segmented genome

Virus-specified hemagglutinin and neuraminidase spikes

Influenza A and B viruses each consist of a nucleocapsid containing eight segments of negative-sense, **single-stranded RNA,** which is enveloped in a glycolipid membrane derived from the host cell plasma membrane. The inner side of the envelope contains a layer of virus-specified protein (M1). Two virus-specified glycoproteins, hemagglutinin and neuraminidase, are embedded in the outer surface of the envelope and appear as "spikes" over the surface of the virion. Figure 33–1 illustrates the makeup of influenza A virus. Influenza B is somewhat similar but has a unique NB protein instead of M2. Influenza C differs from the others in that it possesses only seven RNA segments and has no neuraminidase, although it does possess other receptor-destroying capability (see below). In addition, the hemagglutinin of influenza C binds to a cell receptor different from that for types A and B.

The virus-specified glycoproteins are antigenic and have special functional importance to the virus. **Hemagglutinin** is so named because of its ability to agglutinate red

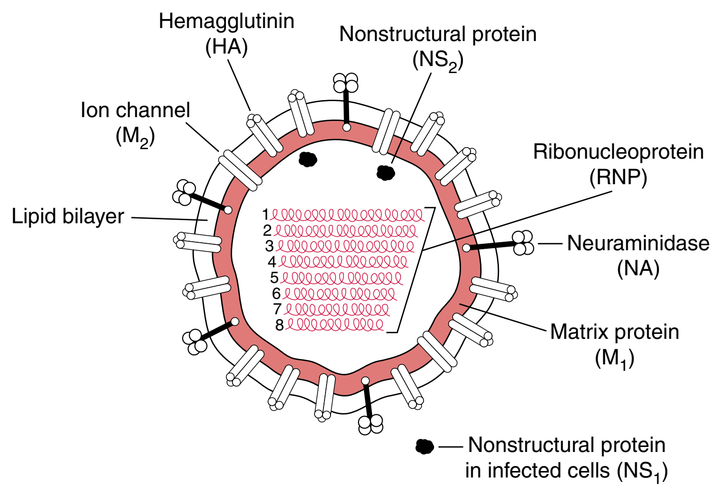


FIGURE 33–1
Diagrammatic view of influenza A virus. Three types of membrane proteins are inserted in the lipid bilayer: hemagglutinin (as trimer), neuraminidase (as tetramer), and M2 ion channel protein. The eight RNP segments each contain viral RNA surrounded by nucleoprotein and associated with RNA transcriptase.

blood cells from certain species (eg, chickens, guinea pigs) in vitro. Its major biological function is to serve as a point of attachment to *N*-acetylneuraminic (sialic) acid–only containing glycoprotein or glycolipid receptor sites on human respiratory cell surfaces, which is a critical first step in initiating infection of the cell.

Hemagglutinin acts in viral attachment

**Neuraminidase** is an antigenic hydrolytic enzyme that acts on the hemagglutinin receptors by splitting off their terminal neuraminic (sialic) acid. The result is destruction of receptor activity. Neuraminidase serves several functions. It may inactivate a free mucoprotein receptor substance in respiratory secretions that could otherwise bind to viral hemagglutinin and prevent access of the virus to the cell surface. It is important in fusion of the viral envelope with the host cell membrane as a prerequisite to viral entry. It also aids in the release of newly formed virus particles from infected cells, thus making them available to infect other cells. Type-specific antibodies to neuraminidase appear to inhibit the spread of virus in the infected host and to limit the amount of virus released from host cells.

Neuraminidase has role in envelope fusion and viral release

**Nucleocapsid** assembly takes place in the cell nucleus, but final virus assembly takes place at the plasma membrane. The ribonucleoproteins are enveloped by the plasma membrane, which by then contains hemagglutinin and neuraminidase. Virus "buds" are formed, and intact virions are released from the cell surface (see Chapter 6, Fig 6–11).

Nucleocapsid and virus assembly occur at different cell sites

Influenza A viruses were initially isolated in 1933 by intranasal inoculation of ferrets, which developed febrile respiratory illnesses. The viruses replicate in the amniotic sac of embryonated hen's eggs, where their presence can be detected by the hemagglutination test. Most strains can also be readily isolated in cell culture systems, such as primary monkey kidney cells. Some cause cytopathic effects in culture.

Viral isolation in eggs or cell cultures

The most efficient method of detection is demonstration of hemadsorption by adherence of erythrocytes to infected cells expressing hemagglutinin or by agglutination of erythrocytes by virus already released into the extracellular fluid. The virus can then be identified specifically by inhibition of these properties by addition of antibody directed specifically at the hemagglutinin. This method is called **hemadsorption inhibition** or **hemagglutination inhibition,** depending on whether the test is performed on infected cells or extracellular virus, respectively. Because the hemagglutinin is antigenic, hemagglutination inhibition tests can also be used to detect antibodies in infected subjects. Research has shown that antibody directed against specific hemagglutinin is highly effective in neutralizing the infectivity of the virus.

Hemadsorption and hemagglutination inhibition used to detect presence of virus

Antihemagglutinin antibodies detectable in serum

## *Influenza A*

Influenza A is considered in detail because of its great clinical and epidemiologic importance.

The influenza A virion contains eight segments of single-stranded RNA with defined genetic responsibilities. These functions include coding for virus-specified proteins (see Fig 33–1; Table 33–2). A unique aspect of influenza A viruses is their ability to develop a wide variety of subtypes through the processes of mutation and whole-gene "swapping" between strains, called **reassortment.** Recombination, which occurs when new genes are assembled from sections of other genes, is thought to occur rarely, if at all. These processes result in antigenic changes called **drifts** and **shifts,** which are discussed shortly.

Influenza A genome in multiple segments

Mutability of virus produces antigenic changes

The 15 recognized subtypes of hemagglutinin and 9 neuraminidase subtypes known to exist among influenza A viruses that circulate in birds and mammals represent a reservoir of viral genes that can undergo reassortment, or "mixing" with human strains. Three hemagglutinins ($H_1$, $H_2$, and $H_3$) and two neuraminidases ($N_1$ and $N_2$) appear to be of greatest importance in human infections. These subtypes are designated according to the H and N antigens on their surface (eg, $H_1N_1$, $H_3N_2$). There may also be more subtle, but sometimes important, antigenic differences (drifts) within each subtype. These differences are designated according to the major representative virus to which they are most closely related antigenically, using the place of initial isolation, number of the isolate, and

Subtypes based on H and N antigens

Subtle changes known as antigenic drift

TABLE 33–2

**Virus-coded Proteins of Influenza A**

| RNA Segment | Proteins | Function |
|---|---|---|
| 1 | PB2 | RNA synthesis, ? virulence |
| 2 | PB1 | RNA synthesis |
| 3 | PA | RNA synthesis |
| 4 | HA | Attachment |
| 5 | NP | RNA synthesis |
| 6 | NA | Virus release from infected cells |
| 7 | M1, M2 | Matrix |
| 8 | NS1, NS2 | Nonstructural; NS1 is interferon antagonist |

year of detection. For example, two $H_3N_2$ strains that differ antigenically only slightly are A/Texas/1/77($H_3N_2$) and A/Bangkok/1/79($H_3N_2$).

Antigenic drift every few years with type A

Antigenic drifts within major subtypes can involve either H or N antigens, as well as the genes encoding nonstructural proteins, and may result from as little as a single mutation in the viral RNA. The mutant may come to predominate under selective immunologic pressures in the host population. Such drifts are frequent among influenza A viruses, occurring at least every few years and sometimes even during the course of a single epidemic. Drifts can also develop in influenza B viruses but at a considerably lower frequency.

Major antigenic shifts due to reassortment

New subtype may also develop mutations

In contrast to the frequently occurring mutations that cause antigenic drift among influenza A strains, major changes (>50%) in the nucleotide sequences of the H or N genes can occur suddenly and unpredictably. These are referred to as antigenic shifts. They almost certainly result from reassortment that can be readily reproduced in the laboratory. Simultaneously infecting a cell with two different influenza A subtypes yields progeny that contain antigens derived from either of the original viruses. For example, a cell infected simultaneously with influenza A ($H_3N_2$) and influenza A ($H_1N_1$) may produce a mixture of influenza viruses of the subtypes $H_3N_2$, $H_1N_1$, $H_1N_2$, and $H_3N_1$. When novel "new" epidemic strains emerge, they most likely have circulated into animal or avian reservoirs where they have undergone genetic reassortment (and sometimes also mutation), then readapted and spread to human hosts when a sufficient proportion of the population has little or no immunity to the "new" subtypes. A recent example was the appearance in Hong Kong in 1997 of human cases caused by an avian influenza A ($H_5N_1$). Studies indicated that all RNA segments were derived from an avian influenza A virus, but a single insert coding for several additional amino acids in the hemagglutinin protein facilitated cleavage by human cellular enzymes. In addition, a single amino acid substitution in the PB2 polymerase protein occurred. These two mutations together made the virus more virulent for humans; fortunately, human-to-human transmission was poor.

Major antigenic shifts correlate with epidemics

Major antigenic shifts, which occurred approximately every 8 to 10 years in the 20th century, often resulted in serious epidemics or pandemics among populations with little or no preexisting antibody to the new subtypes. Examples include the appearance of an $H_1N_1$ subtype in 1947, followed by an abrupt shift to an $H_2N_2$ strain in 1957, which caused the pandemic of Asian flu. A subsequent major shift in 1968 to an $H_3N_2$ subtype (the Hong Kong flu) led to another, but somewhat less severe epidemic. The Russian flu, which appeared in late 1977, was caused by an $H_1N_1$ subtype very similar to that which dominated between 1947 and 1957 (Table 33–3).

Minor antigenic drifts allow maintenance in population

The concepts of antigenic shift and drift in human influenza A virus infections can be approximately summarized as follows. Periodic shifts in the major antigenic components appear, usually resulting in major epidemics in populations with little or no immunologic

TABLE 33–3

**Major Antigenic Shifts Associated with Influenza A Pandemics, 1947–1987**

| Year | Subtype | Prototype Strain |
|---|---|---|
| 1947 | $H_1N_1$ | A/FM1/47 |
| 1957 | $H_2N_2$ | A/Singapore/57 |
| 1968 | $H_3N_2$ | A/Hong Kong/68 |
| 1977 | $H_1N_1$ | A/USSR/77 |
| 1987 | $H_3N_2$ | No pandemic occurred; various strains circulating worldwide |

experience with the subtype. As the population of susceptible individuals is exhausted (ie, subtype-specific immunity is acquired by increasing numbers of people), the subtype continues to circulate for a time, undergoing mutations with subtle antigenic drifts from season to season. This allows some degree of virus transmission to continue. Infectivity persists because subtype-specific immunity is not entirely protective against drifting strains; for example, an individual may have antibodies reasonably protective against influenza A/Texas/77($H_3N_2$), yet be susceptible in succeeding years to reinfection by influenza A/Bangkok/79($H_3N_2$). Eventually, however, the overall immunity of the population becomes sufficient to minimize the epidemic potential of the major subtype and its drifting strains. Unfortunately, the battle is never entirely won; the scene is set for the sudden and usually unpredictable appearance of an entirely new subtype that may not have circulated among humans for 20 years or more.

Individual variation is significant

## INFLUENZA

Clinical Capsule

Influenza virus types A and B typically cause more severe symptoms than influenza virus type C. The typical illness is characterized by an abrupt onset (over several hours) of fever, diffuse muscle aches and chills. This is followed within 12 to 36 hours by respiratory signs, such as rhinitis, cough, and respiratory distress. The acute phase usually lasts 3 to 5 days, but a complete return to normal activities may take 2 to 6 weeks. Serious complications, especially pneumonia, are common.

## EPIDEMIOLOGY

Humans are the major hosts of the influenza viruses, and severe respiratory disease is the primary manifestation of infection. However, influenza A viruses closely related to those prevalent in humans circulate among many mammalian and avian species. As noted previously, some of these may undergo antigenic mutation or genetic recombination and emerge as new human epidemic strains.

Human, animal, and avian strains are similar

Characteristic influenza outbreaks have been described since the early 16th century, and outbreaks of varying severity have occurred nearly every year. Severe pandemics occurred in 1743, 1889–1890, 1918–1919 (the Spanish flu), and 1957 (the Asian flu). These episodes were associated with particularly high mortality; the Spanish flu was thought to have caused at least 20 million deaths. Usually, the elderly and persons of any age group with cardiac or pulmonary disease have the highest death rate.

Pandemic influenza may have high mortality

**Direct droplet spread** is the most common mode of transmission. Influenza infections in temperate climates tend to occur most frequently during midwinter months. Major epidemics of influenza A usually occur at 2- to 3-year intervals, and influenza

Seasonality favors winter months

Epidemic intervals usually a few years

Excess mortality or increased absenteeism are indictors of epidemics

B epidemics occur irregularly, usually every 4 to 5 years. The typical epidemic develops over a period of 3 to 6 weeks and can involve 10% of the population. Illness rates may exceed 30% among school-aged children, residents of closed institutions, and industrial groups. One major indicator of influenza virus activity is an abrupt rise in school or industrial absenteeism. In severe influenza A epidemics, the number of deaths reported in a given area of the country often exceeds the number expected for that period. This significant increase, referred to as **excess mortality,** is another indicator of severe, widespread illness. Influenza B rarely causes such severe epidemics.

## PATHOGENESIS

Virus multiplies in respiratory epithelium

Synthetic blocks cause cilial damage and cell desquamation

Clearance mechanisms compromised

Influenza viruses have a predilection for the respiratory tract, and viremia is rarely detected. They multiply in ciliated respiratory epithelial cells, leading to functional and structural ciliary abnormalities. This is accompanied by a switch-off of protein and nucleic acid synthesis in the affected cells, the release of lysosomal hydrolytic enzymes, and desquamation of both ciliated and mucus-producing epithelial cells. Thus, there is substantial interference with the mechanical clearance mechanism of the respiratory tract. The process of programmed cell death (apoptosis) results in the cleavage of complement components, leading to localized inflammation. Early in infection, the primary chemotactic stimulus is directed toward mononuclear leukocytes, which constitute the major cellular inflammatory component. The respiratory epithelium may not be restored to normal for 2 to 10 weeks after the initial insult.

Viral toxicity causes inflammation

Phagocytic host defenses compromised

The virus particles are also toxic to tissues. This toxicity can be demonstrated by inoculating high concentrations of inactivated virions into mice, which produces acute inflammatory changes in the absence of viral penetration or replication within cells. Other host cell functions are also severely impaired, particularly during the acute phase of infection. These functions include chemotactic, phagocytic, and intracellular killing functions of polymorphonuclear leukocytes and perhaps of alveolar macrophage activity.

Damage creates susceptibility to bacterial invasion

Interferon and cytotoxic T-cell responses associated with recovery

The net result of these effects is that, on entry into the respiratory tract, the viruses cause cell damage, especially in the respiratory epithelium, which elicits an acute inflammatory response and impairs mechanical and cellular host responses. This damage renders the host highly susceptible to invasive bacterial **superinfection.** In vitro studies also suggest that bacterial pathogens such as staphylococci can more readily adhere to the surfaces of influenza virus-infected cells. Recovery from infection begins with interferon production, which limits further virus replication, and with rapid generation of natural killer cells. Shortly thereafter, class I major histocompatibility complex (MHC)–restricted cytotoxic T cells appear in large numbers to participate in the lysis of virus-infected cells and, thus, in initial control of the infection. This is followed by the appearance of local and humoral antibody along with an evolving, more durable cellular immunity. Finally, there is repair of tissue damage.

## IMMUNITY

Antihemagglutinin antibody has protective effect

Antineuraminidase may limit viral spread

Although cell-mediated immune responses are undoubtedly important in influenza virus infections, humoral immunity has been investigated more extensively. Typically, patients respond to infection within a few days by producing antibodies directed toward the group ribonucleoprotein antigen, the hemagglutinin, and the neuraminidase. Peak antibody titer levels are usually reached within 2 weeks of onset and then gradually wane over the following months to varying low levels. Antibody to the ribonucleoprotein appears to confer little or no protection against reinfection. Antihemagglutinin antibody is considered the most protective; it has the ability to neutralize virus on reexposure. However, such immunity is relative, and quantitative differences in responsiveness exist between individuals. Furthermore, antigenic shifts and drifts often allow the virus to subvert the antibody response on subsequent exposures. Antibody to neuraminidase antigen is not as protective as antihemagglutinin antibody but plays a role in limiting virus spread within the host.

# INFLUENZA: CLINICAL ASPECTS

## MANIFESTATIONS

As stated previously, influenza A and B viruses tend to cause the most severe illnesses, whereas influenza C seems to occur infrequently and generally causes milder disease. The typical acute influenzal syndrome is described here.

The incubation period is brief, lasting an average of 2 days. Onset is usually abrupt, with symptoms developing over a few hours. These include fever, myalgia, headache, and occasionally shaking chills. Within 6 to 12 hours, the illness reaches its maximum severity, and a dry, nonproductive cough develops. The acute findings persist, sometimes with worsening cough, for 3 to 5 days, followed by gradual improvement. By about 1 week after onset, patients feel significantly better. However, fatigue, nonspecific weakness, and cough can remain frustrating lingering problems for an additional 2 to 6 weeks.

Short incubation period followed by acute disease with dry cough

Occasionally, patients develop a progressive infection that involves the tracheobronchial tree and lungs. In these situations, pneumonia, which can be lethal, is the result. Other unusual acute manifestations of influenza include central nervous system (CNS) dysfunction, myositis, and myocarditis. In infants and children, a serious complication known as Reye's syndrome may develop 2 to 12 days after onset of the infection. It is characterized by severe fatty infiltration of the liver and cerebral edema. This syndrome is associated not only with influenza viruses but with a wide variety of systemic viral illnesses. The risk is enhanced by exposure to salicylates, such as aspirin.

Progressive respiratory infection and pneumonia may be lethal

Reye's syndrome may follow

The most common and important complication of influenza virus infection is bacterial superinfection. Such infections usually involve the lung, but bacteremia with secondary seeding of distant sites can also occur. The superinfection, which can develop at any time in the acute or convalescent phase of the disease, is often heralded by an abrupt worsening of the patient's condition after initial stabilization. The bacteria most commonly involved include *Streptococcus pneumoniae, Haemophilus influenzae,* and *Staphylococcus aureus.*

Sudden worsening suggests bacterial superinfection

In summary, there are essentially three ways in which influenza may cause death:

- **Underlying disease with decompensation.** Individuals with limited cardiovascular or pulmonary reserves can be further compromised by any respiratory infection. Thus, the elderly and those of any age with underlying chronic cardiac or pulmonary disease are at particular risk.
- **Superinfection.** Superinfection can lead to bacterial pneumonia and occasionally disseminated bacterial infection.
- **Direct rapid progression.** Less commonly, progression of the viral infection can lead to overwhelming viral pneumonia with asphyxia.

## DIAGNOSIS

During the acute phase of illness, influenza viruses can be readily isolated from respiratory tract specimens, such as nasopharyngeal and throat swabs. Most strains grow in primary monkey kidney cell cultures, and they can be detected by hemadsorption or hemagglutination. Rapid diagnosis of infection is possible by direct immunofluorescence or immunoenzymatic detection of viral antigen in epithelial cells or secretions from the respiratory tract. Serologic diagnosis is of considerable help epidemiologically and is usually made by demonstrating a fourfold or greater increase in hemagglutination inhibition antibody titers in acute and convalescent specimens collected 10 to 14 days apart.

Virus isolation detects virus

Rapid detection of antigen often used

Serodiagnosis is useful epidemiologically

## TREATMENT

The two basic approaches to management of influenzal disease are symptomatic care and anticipation of potential complications, particularly bacterial superinfection. Once the diagnosis has been made, rest, adequate fluid intake, conservative use of analgesics for

Supportive therapy indicated

TABLE 33–4

**Comparison of Antiviral Drugs for Influenza**

| Feature | Amantadine | Rimantadine | Zanamivir | Oseltamivir |
|---|---|---|---|---|
| Susceptible viruses | Influenza A only | Influenza A only | Influenza A and B | Influenza A and B |
| Emergent resistant strains | Yes | Yes | Not known | Not known |
| Administration | Oral | Oral | Inhalation | Oral |

myalgia and headache, and antitussives for severe cough are commonly prescribed. It must be emphasized that nonprescription drugs must be used with caution. This applies particularly to drugs containing salicylates given to children, because the risk of Reye's syndrome must be considered.

Antibiotic prophylaxis does not prevent bacterial superinfection

Bacterial superinfection is often suggested by a rapid worsening of clinical symptoms after patients have initially stabilized. Antibiotic prophylaxis has not been shown to enhance or diminish the likelihood of superinfection but can increase the risk of acquisition of more resistant bacterial flora in the respiratory tract and make the superinfection more difficult to treat. Ideally, physicians should instruct patients regarding the natural history of the influenza virus infection and be prepared to respond to bacterial complications, if they occur, with specific diagnosis and therapy.

Antiviral therapy must begin early

When influenza A infection is proved or strongly suspected, 4 to 5 days of therapy with amantadine or rimantadine, two symmetric amines, may also be considered (Table 33–4). Such treatment has been shown to benefit some patients to a modest degree, as measured by reduction of number of days of confinement to bed, of fever, and of functional respiratory impairment. However, these effects have been observed only when the drug is administered early in the illness (within 12 to 24 hours of onset). The neuraminidase inhibitors (zanamivir or oseltamivir) have also proved beneficial, if begun early. They are also active against influenza B (see Table 33–4).

## PREVENTION

Whole virus and "split" vaccines are protective but variable and of short duration

Annual revaccination against most current strains is necessary

Vaccination indicated for high-risk individuals

The best available method of control is by use of **killed viral vaccines** newly formulated each year to most closely match the influenza A and B antigenic subtypes currently causing infections. These inactivated vaccines may contain whole virions or "split" subunits composed primarily of hemagglutinin antigens. They are commonly used, in two doses given 1 month apart, to immunize children who may not have been immunized previously; among older children and adults, single annual doses are recommended just prior to influenza season. Vaccine efficacy is variable, and annual revaccination is necessary to ensure maximal protection. Used in this way, the virus vaccines may be 70 to 85% effective. It is recommended that vaccination be directed primarily toward the elderly, individuals of all ages who are at high risk (eg, those with chronic lung or heart disease), and their close contacts, including medical personnel and household members. Live attenuated vaccines that are administered by nose drops are also being evaluated, and show considerable promise for the future.

Amantadine or rimantadine prophylaxis effective short-term for influenza A only

Blocks virus uncoating and assembly

Studies have shown that both amantadine and rimantadine are effective in short-term (several weeks) oral prophylaxis of influenza A infections. They act by blocking the ion channel of the viral M2 protein, resulting in interference with the key role for M2 protein in early virus uncoating. Later virion assembly may also be affected. However, these agents have side effects and are recommended only for high-risk patients until vaccine-induced immunity can be achieved. A typical example of their use would be during an epidemic in which an elderly, potentially susceptible patient may become exposed to infection within a defined period. Oral prophylaxis may be initiated concurrently with administration of a vaccine containing the most current antigens and continued for 2 weeks. The immunogenic effect of the vaccine should ensure continued protection. It must be emphasized that these drugs have been proven effective for influenza A virus infections only; they are useless in the management and prevention of infections caused by other types of influenza or by any other respiratory virus. Unfortunately, virus resistance to both drugs can readily develop in vitro or in vivo. A single amino acid substitution in the transmembrane portion of the M2 protein is all that is necessary for this to occur.

Resistance from single amino acid substitution in M2 protein

Zanamivir and oseltamivir, approved for use in 1999, both act by blocking the enzymatically active neuraminidase glycoprotein present on the surfaces of influenza A and B viruses, thus limiting virus release from infected cells, and subsequent spread in the host. No viral resistance to these drugs has yet been noted (see Table 33–4).

Neuraminidase inhibitors are useful for influenza A and B

# RESPIRATORY SYNCYTIAL VIRUS

## VIROLOGY

Respiratory syncytial virus (RSV) is classified as a pneumovirus within the paramyxovirus family. Its name is derived from its ability to produce cell fusion in tissue culture (syncytium formation). Unlike influenza or parainfluenza viruses, it possesses no hemagglutinin or neuraminidase. The RNA genome is nonsegmented, negative sense, and single stranded and codes for at least 10 different proteins. Among these are two matrix (M) proteins in the viral envelope. One forms the inner lining of the viral envelope; the function of the other is uncertain.

Pneumovirus causing syncytium formation in cell cultures

Enveloped RNA virus with unsegmented genome

The antigens on the surface spikes of the viral envelope include the G glycoprotein, which mediates virus attachment to host cell receptors, and the fusion (F) glycoprotein, which induces fusion of the viral envelope with the host cell surface to facilitate entry. F glycoprotein is also responsible for fusion of infected cells in cell cultures, leading to the appearance of multinucleated giant cells (syncytium formation). Antibodies directed at the F glycoprotein are more efficient than anti-G glycoprotein antibodies in neutralizing the virus in vitro.

Two glycoproteins mediate attachment and syncytium formation

At least two antigenic subgroups (A and B) of RSV are known to exist. This dimorphism is due primarily to differences in the G glycoprotein. The epidemiologic and biological significance of these variants is not yet certain; however, epidemiologic studies have suggested that group A infections tend to be more severe. RSV is the single most important etiologic agent in respiratory diseases of infancy, and it is the major cause of bronchiolitis and pneumonia among infants under 1 year of age.

RSV is most important respiratory virus in infants

## RESPIRATORY SYNCYTIAL VIRUS DISEASE

CLINICAL CAPSULE

RSV primarily infects the bronchi, bronchioles, and alveoli of the lung. The illnesses clinically categorized as croup, bronchitis, bronchiolitis or pneumonia are extremely common in infants. The acute phase of cough, wheezing and respiratory distress lasts 1 to 3 weeks. The severity of respiratory involvement and the high prevalence during outbreaks both account for a large number of hospitalizations on pediatric units each year. Elderly or immunocompromised patients are also frequently susceptible and can be severely affected.

### EPIDEMIOLOGY

Community outbreaks of RSV infection occur annually commencing at any time from late fall to early spring. The usual outbreak lasts 8 to 12 weeks and can involve nearly one half of all families with children. In the family setting, it appears that older siblings often introduce the virus into the home, and secondary infection rates can be almost 50%. The usual duration of virus shedding is 5 to 7 days; young infants, however, may shed virus for 9 to 20 days or longer.

High attack rate, introduced by older siblings

Nosocomial infection reduced by careful handwashing

Spread of RSV in the hospital setting is also a major problem. Control is difficult, but includes careful attention to handwashing between contacts with patients, isolation, and exclusion of personnel and visitors who have any form of respiratory illness. Masks are not effective in controlling nosocomial spread.

## PATHOGENESIS

Confined to respiratory epithelium

RSV is spread to the upper respiratory tract by contact with infective secretions. Infection appears to be confined primarily to the respiratory epithelium, with progressive involvement of the middle and lower airways. Viremia occurs rarely. The direct effect of virus on respiratory tract epithelial cells is similar to that previously described for influenza viruses, and cytotoxic T cells appear to play a similar role in early control of the acute infection.

Enhanced disease in infants may have immunologic basis

$Th_2$ stimulated cytokines cause injury

The apparent enhanced severity of disease, particularly in very young infants, is not yet clearly understood but may have an immunologic basis. Factors that have been proposed to play a role include (1) qualitative or quantitative deficits in humoral or secretory antibody responses to critical virus-specified proteins, (2) formation of antigen–antibody complexes within the respiratory tract resulting in complement activation, or (3) excessive damage from inflammatory cytokines. Experimental evidence suggests that patients who respond to RSV infections with CD4+ cells that are predominantly of the $T_H$ type 2 have more severe disease than those with predominant $T_H$ type 1 responses. This is thought to be due to the inflammatory cytokines produced by $T_H$ type 2 cells, including interleukin (IL)-4, IL-5, IL-6, IL-10, and IL-13.

Necrosis and inflammation plugs bronchioles and alveoli

The major pathologic findings are in the bronchi, bronchioles, and alveoli. These include necrosis of epithelial cells; interstitial mononuclear cell inflammatory infiltrates, which sometimes also involve the alveoli and alveolar ducts; and plugging of smaller airways with material containing mucus, necrotic cells, and fibrin (Fig 33–2). Multinucleated syncytial cells with intracytoplasmic inclusions are occasionally seen in the affected tracheobronchial epithelium.

## IMMUNITY

Immunity to reinfection is brief

Infection results in IgG and IgA humoral and secretory antibody responses. However, immunity to reinfection is quite tenuous, as demonstrated by patients who have recovered from a primary acute episode and have become reinfected with disease of similar severity in the same or succeeding year. Illness severity appears to diminish with increasing age and successive reinfection.

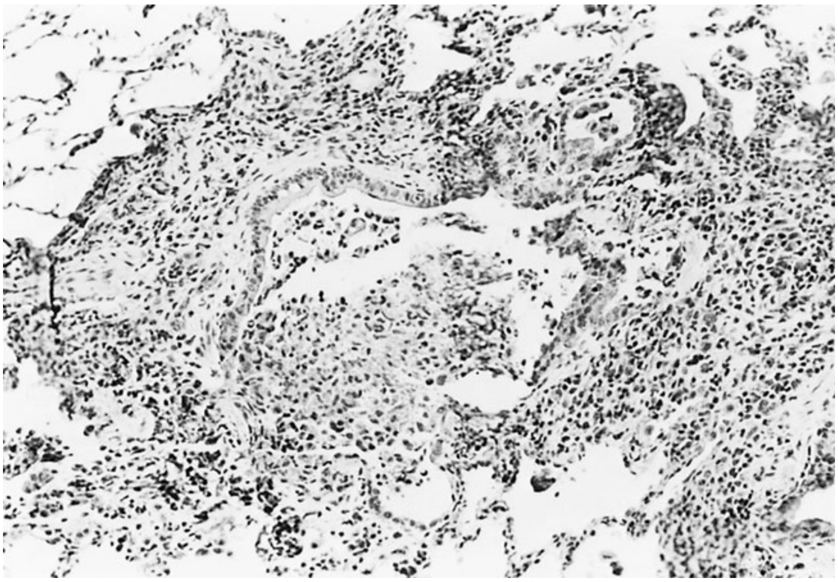

FIGURE 33–2
Photomicrograph illustrating the bronchiolar and surrounding interstitial inflammation in respiratory syncytial virus infection. (Original magnification ×100.)

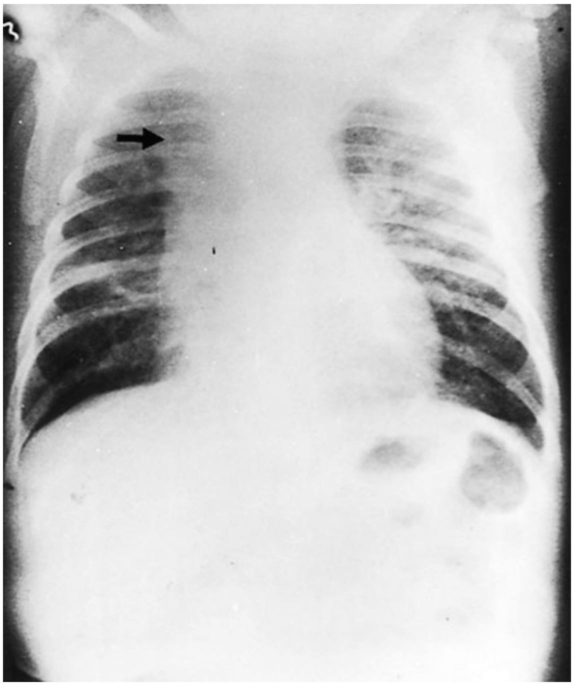

FIGURE 33–3
Chest radiograph of an infant with a severe case of respiratory syncytial virus pneumonia and bronchiolitis. Bilateral interstitial infiltrates, hyperexpansion of the lung, and right upper lobe atelectasis (arrow) are all present.

## RESPIRATORY SYNCYTIAL VIRUS DISEASE: CLINICAL ASPECTS

### MANIFESTATIONS

The usual incubation period is 2 to 4 days, followed by the onset of rhinitis; severity of illness progresses to a peak within 1 to 3 days. In infants, this peak usually takes the form of bronchiolitis and pneumonitis, with cough, wheezing, and respiratory distress. Clinical findings include **hyperexpansion** of the lungs, **hypoxemia** (low oxygenation of blood), and **hypercapnia** (carbon dioxide retention). Interstitial infiltrates, often with areas of pulmonary collapse, may be seen on chest radiography (Fig 33–3). Fever is variable. The duration of acute illness is often 10 to 14 days.

Infant bronchiolitis and pneumonitis lasts up to 2 weeks

The fatality rate among hospitalized infected infants is estimated to be 0.5 to 1%; however, this rises to 15% or greater in children receiving cancer chemotherapy, infants with congenital heart disease, and those with severe immunodeficiency. Infants with underlying chronic lung disease are also at high risk. Causes of death include respiratory failure, right-sided heart failure (cor pulmonale), and bacterial superinfection. Death has sometimes resulted from unnecessary procedures in patients in whom RSV infection was not considered. Bronchoscopy, lung biopsy, or overly aggressive therapy with corticosteroids and bronchodilators for presumed asthma can all pose a danger to such patients.

Mortality is highest with underlying disease

Older infants, children, and adults are also readily infected. The clinical illnesses in these groups are usually milder and include croup, tracheobronchitis, and upper respiratory infection (URI); however, elderly persons can experience severe morbidity. RSV can also cause acute flare-ups of chronic bronchitis and trigger acute wheezing episodes in asthmatic children.

Children and adults have milder illness

Can trigger wheezing in asthmatics

### DIAGNOSIS

Rapid diagnosis of RSV infection can be made by immunofluorescence or immunoenzyme detection of viral antigen. The virus can also be isolated from the respiratory tract by prompt inoculation of specimens into cell cultures. Syncytial cytopathic effects

Virus isolation, immunofluorescence, or immunoassay detect RSV develop over 2 to 7 days. Serodiagnosis may also be used but requires acute and convalescent sera and is less sensitive than antigen detection methods or culture.

### TREATMENT AND PREVENTION

Supportive treatment is indicated

Treatment is directed primarily at the underlying pathophysiology and includes adequate oxygenation, ventilatory support when necessary, and close observation for complications such as bacterial superinfection and right-sided heart failure. Some studies suggest that ribavirin aerosol treatment may be effective in selected circumstances.

Monoclonal antibody and immune globulin used for prophylaxis

No vaccine is currently available. Attenuated live virus vaccines and immune globulin containing high antibody titers to RSV are also under active investigation; a high-titered monoclonal antibody against F protein has been used for prophylaxis in high-risk infants (those born prematurely or with chronic lung disease). This method requires monthly injections during the RSV season (usually 5 months) and is extremely expensive.

# PARAINFLUENZA VIRUSES

## VIROLOGY

Enveloped paramyxoviruses have neuraminidase and hemagglutinin

Four serotypes are antigenically stable

There are four serotypes of parainfluenza viruses: parainfluenza 1, 2, 3, and 4. These enveloped viruses belong to the paramyxovirus group; contain nonsegmented, negative-sense, single-stranded RNA; and, like the influenza viruses, possess a neuraminidase and hemagglutinin. Their mode of spread and pathogenesis is similar to that of the influenza viruses. They differ from the influenza viruses in that RNA synthesis occurs in the cytoplasm rather than the nucleus. In addition, the antigenic makeup of the four serotypes is relatively stable, and significant antigenic shift or drift does not occur. Each serotype is considered separately.

## PARAINFLUENZA DISEASE

Transient immunity

The parainfluenza viruses are important because of the serious diseases they can cause in infants and young children. Parainfluenza 1 and 3 are particularly common in this regard. Overall, the group is thought to be responsible for 15 to 20% of all nonbacterial respiratory diseases requiring hospitalization in infancy and childhood. Immunity to reinfection is transient; although repeated infections can occur in older children and adults, they are usually milder than the illnesses of infancy and early childhood.

## PARAINFLUENZA DISEASE: CLINICAL ASPECTS

### MANIFESTATIONS

The onset of illness may be abrupt, as in acute spasmodic croup, but usually begins as a mild URI with variable progression over 1 to 3 days to involvement of the middle or lower respiratory tract. Duration of acute illness can vary from 4 to 21 days but is usually 7 to 10 days.

#### Parainfluenza 1

Croup and tracheobronchitis are seen

Parainfluenza 1 is the major cause of acute croup (laryngotracheitis) in infants and young children but also causes less severe diseases such as mild upper respiratory illness (URI), pharyngitis, and tracheobronchitis in individuals of all ages. Outbreaks of infection tend to occur most frequently during the fall months.

### Parainfluenza 2

Parainfluenza 2 is of slightly less significance than parainfluenza 1 or 3. It has been associated with croup, primarily in children, with mild URI, and occasionally with acute lower respiratory disease. As with parainfluenza 1, outbreaks usually occur during the fall months.

Croup is primary disease

### Parainfluenza 3

Parainfluenza 3 is a major cause of severe lower respiratory disease in infants and young children. It often causes bronchitis, pneumonia, and croup in children less than 1 year of age. In older children and adults, it may cause URI or tracheobronchitis. Infections are common and can occur in any season; it is estimated that nearly one half of all children have been exposed to this virus by 1 year of age.

Produces severe lower respiratory disease in infants

### Parainfluenza 4

Parainfluenza 4 is the least common of the group. It is generally associated with mild upper respiratory illness only.

Causes only URI

### DIAGNOSIS, TREATMENT, AND PREVENTION

Specific diagnosis is based on virus isolation, usually in monkey kidney cell cultures, or on serology using hemagglutination inhibition, complement fixation, or neutralization assays on paired sera to detect a rising antibody titer. Immunofluorescence or immunoenzyme assays can also be used for rapid detection of antigen in respiratory epithelial cells. Currently, there is no method of control or specific therapy for these infections.

Laboratory diagnosis by isolation or antigen detection

Croup or URI are not treatable

# ADENOVIRUSES

## VIROLOGY

Of the almost 100 different serotypes of adenoviruses, 49 are known to affect humans. These viruses are naked and icosahedral and possess double-stranded DNA. Replication and assembly occur in the nucleus, and virions are released by cell destruction. All adenoviruses share a common group-specific, complement-fixing antigen associated with the hexon component of the viral capsid. Adenoviruses are characterized by their ubiquity and persistence in host tissues for periods ranging from a few days to several years. Their ability to produce infection without disease is illustrated by the frequent recovery of virus from tonsils or adenoids removed from healthy children (the group name is derived from its discovery in 1953 as a latent agent in many adenoid tissue specimens) and by prolonged intermittent shedding of virus from the pharynx and intestinal tract after initial infection.

Multiple serotypes of naked, double-stranded DNA viruses

Potential for prolonged infection without disease

## ADENOVIRUS DISEASE

### EPIDEMIOLOGY

Type 1 and 2 adenoviruses are highly endemic; type 5 is the next most common. Most primary infections with these viruses occur early in life and are spread by the respiratory or fecal–oral route. Overall, only about 45% of adenovirus infections result in disease. Their most significant contribution to acute illness is in children, particularly those under 2 years of age (approximately 10% of acute febrile illness). Adenoviruses are also major causes of acute respiratory disease in military recruits, usually by types 4 and 7.

Disease in children and military recruits is spread by respiratory or fecal–oral route

Swimming pool and medication-associated conjunctivitis occur in outbreaks

Infections caused by serotypes 1, 2, and 5 are generally most frequent during the first few years of life. All serotypes can occur during any season of the year but are encountered most frequently during late winter or early spring. Sharp outbreaks of disease caused by serotypes 3 and 7 have been traced to inadequately chlorinated swimming pools. Conjunctivitis is the illness most commonly associated with these episodes. Other outbreaks of conjunctivitis have been traced to physicians' offices and appear to have been spread by contaminated ophthalmic medications or diagnostic equipment.

## PATHOGENESIS

Infects by droplet, oral route, or direct inoculation

Epithelial cell replication may be followed by viremic spread and remote disease

The adenoviruses usually enter the host by inhalation of droplet nuclei or by the oral route. Direct inoculation onto nasal or conjunctival mucosa by hands, contaminated towels, or ophthalmic medications may also occur. The virus replicates in epithelial cells, producing cell necrosis and inflammation. Viremia sometimes occurs and can result in spread to distant sites, such as the kidney, bladder, liver, lymphoid tissue (including mesenteric nodes), and, occasionally, the CNS. In the acute phase of infection, the distant sites may also show inflammation; for example, abdominal pain is occasionally seen with severe illnesses and is believed to result from mesenteric lymphadenitis caused by the viruses.

Integration of adenoviral DNA produces latency

After the acute phase of illness, the viruses may remain in tissues, particularly lymphoid structures such as tonsils, adenoids, and intestinal Peyer's patches, and become reactivated and shed without producing illness for 6 to 18 months thereafter. This reactivation is enhanced by stressful events (stress reactivation), such as infection by other agents. Integration of adenoviral DNA into the host cell genome has been shown to occur; this latent state can persist for years in tonsillar tissue and peripheral blood lymphocytes.

Penton projections are toxic to cells

Like the viruses described previously, adenoviruses have a primary pathology involving epithelial cell necrosis with a predominantly mononuclear inflammatory response. In some instances, smudgy intranuclear inclusions may be seen in infected cells (Fig 33–4). A potentially important pathogenic feature of the virion is the presence of pentons, which are located at each of the 12 corners of the icosahedron. These fiber-like projections with knob-like terminal structures are believed to bind to a cellular receptor that is similar or identical to the one for group B coxsackieviruses. The pentons also appear to be responsible for a toxic effect on cells, which manifests as clumping and detachment in vitro.

Proteins restrict cytotoxic T cells and enhance cytokine susceptibility

In addition, adenoviruses have developed other novel strategies to survive in the host yet produce deleterious effects. These include encoding a protein in its early E3 genomic region that binds class I MHC antigens in the endoplasmic reticulum, thus restricting their expression on the surface of infected cells and interfering with recognition and attack by cytotoxic T cells. This ability to evade immunosurveillance may be vital to establishment of latency. Another early protein (E1A) has been associated with increased susceptibility

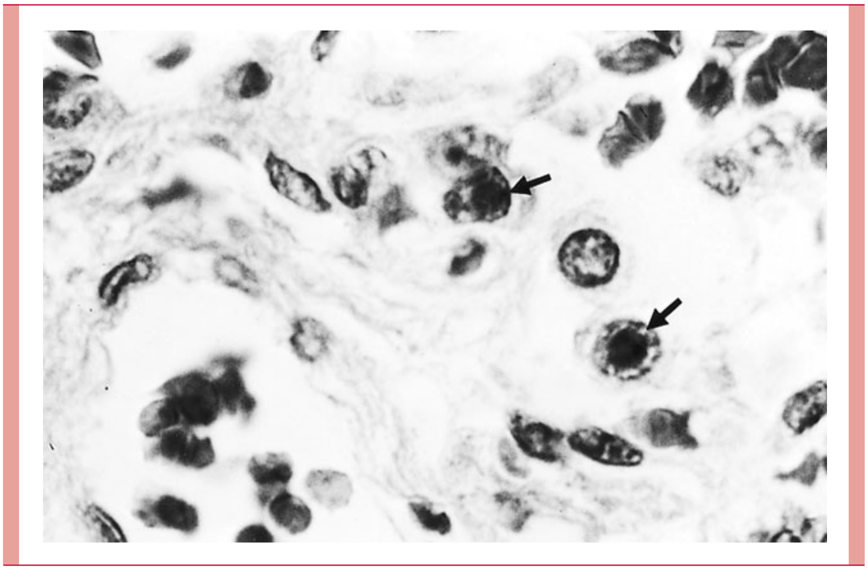

FIGURE 33–4
Lung tissue from a fatal case of adenovirus type 7 pneumonia. Large, smudgy intranuclear inclusions in alveolar epithelial cells (arrows), which are sometimes seen in adenovirus infections, are present. (Original magnification ×100.)

of epithelial cells to destruction by tumor necrosis factor and other cytokines. Other adenoviral proteins have been described that have a variety of effects on cell function and susceptibility to cytolysis. One of these, called the adenovirus death protein, is considered important for efficient lysis of infected cells and release of newly formed virions.

## IMMUNITY

Immunity after infection is serotype specific and usually long lasting. In addition to type-specific immunity, group-specific complement-fixing antibodies appear in response to infection. These antibodies are useful indicators of infection, but do not specify the infecting serotype.

Immunity is type specific

# ADENOVIRUS DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

The diversity of major syndromes and serotypes commonly associated with adenoviruses are summarized in Table 33–5. The acute respiratory syndromes vary in both clinical manifestations and severity. Symptoms include fever, rhinitis, pharyngitis, cough, and conjunctivitis. Adenoviruses are also common causes of nonstreptococcal exudative pharyngitis, particularly among children less than 3 years of age. Acute, and occasionally chronic, conjunctivitis and keratoconjunctivitis have been associated with several serotypes. More severe disease, such as laryngitis, croup, bronchiolitis, and pneumonia, may also occur. A syndrome of pharyngitis and conjunctivitis (pharyngoconjunctival fever) is classically associated with adenovirus infection. Adenoviruses can also cause acute hemorrhagic cystitis, in which hematuria and dysuria are prominent findings. Some serotypes are significant causes of gastroenteritis (see Chapter 39).

Multiple upper respiratory syndromes, conjunctivitis, and pharyngitis are common

More severe disease includes hemorrhagic cystitis

## DIAGNOSIS

Many serotypes, other than those associated with acute gastroenteritis, can be readily isolated in heteroploid cell cultures. There is little difficulty in relating the virus detected to the illness in question when the isolate has been obtained from a site other than the upper respiratory or gastrointestinal tract (eg, lung biopsy, conjunctival swabs, urine). However, because of the known tendency for intermittent asymptomatic shedding into the oropharynx and feces, isolates from these latter sites must be interpreted more cautiously. Serologic testing of acute and convalescent sera may be necessary to confirm the relationship between the virus and the illness in question.

Viral isolation from oropharynx or feces may not mean disease

TABLE 33–5

**Clinical Syndromes Associated with Adenovirus Infection**

| Syndrome | Common Serotypes[a] |
|---|---|
| Childhood febrile illness; pharyngoconjunctival fever | 1, 2, **3,** 5, 7, **7a** |
| Pneumonia and other acute respiratory illnesses | 1, 2, **3**, 5, 7, **7a**, **7b** (4 in military recruits) |
| Pertussis-like illness | 1, 2, **3**, 5, **19**, 21 |
| Conjunctivitis | 2, 5, 7, 8, **19**, 21 |
| Keratoconjunctivitis | **3**, 8, 9, **19** |
| Acute hemorrhagic cystitis | 11 |
| Acute gastroenteritis | 40, 41 |

[a]Serotypes in **boldface** are those commonly associated with outbreaks.

## TREATMENT AND PREVENTION

Live vaccine used in military

There is no specific therapy for infection. A live virus vaccine containing serotypes 4 and 7, enclosed in enteric-coated capsules and administered orally, has been used in military recruits. The viruses are released into the small intestine, where they produce an asymptomatic, nontransmissible infection. This vaccine has been found effective but is neither available nor recommended for civilian groups.

# RHINOVIRUSES

## VIROLOGY

Small, naked RNA viruses include multiple serotypes

Optimum growth temperature is 33°C

Virus binds to ICAM intercellular adhesion molecule

The rhinovirus group comprises at least 115 accepted serotypes and more that are not yet classified. These picornaviruses are small (20 to 30 nm), naked particles containing single-stranded, positive-sense RNA. They are distinguished from enteroviruses by their acid lability and an optimum temperature of 33°C for in vitro replication. This temperature approximates that of the nasopharynx in the human host and may be a factor in the localization of pathologic findings at that site. Rhinoviruses are most consistently isolated in cultures of human diploid fibroblasts. The receptor for most rhinoviruses (and some coxsackieviruses) is glycoprotein intercellular adhesion molecule 1 (ICAM-1), a member of the immunoglobulin supergene family. ICAM-1 is best known for its role in immunologic cell adhesion; its ligand is lymphocyte function-associated antigen-1.

## RHINOVIRUS DISEASE

Common cold viruses cause mild URI

Minimal cell injury is produced

Rhinoviruses are known as the common cold viruses. They represent the major causes of mild URI syndromes in all age groups, especially older children and adults. Lower respiratory tract disease caused by rhinoviruses is uncommon. The usual incubation period is 2 to 3 days, and acute symptoms commonly last 3 to 7 days. Interestingly, mucosal cell damage is minimal during the illness. Data suggest that activation and an increase in kinins, particularly bradykinin, may have a major role in the pathogenesis of increased secretions, vasodilation, and sore throat. Rhinovirus infections may be seen at any time of the year. Epidemic peaks tend to occur in the early fall or spring months.

## RHINOVIRUS DISEASE: TREATMENT AND PREVENTION

Multiple serotypes make vaccine different

Pharmaceutical agents block attachment to ICAM

Currently there is no specific therapy and no methods of prevention with vaccines. Prospects for the development of an appropriate vaccine appear dim. The multiplicity of serotypes and their tendency to be type specific in the production of antibodies seem to demand the development of a multivalent vaccine, which would be extremely difficult to accomplish. However, recent studies have suggested that a monoclonal antibody directed at the virus receptor or the use of a recombinant soluble receptor (ICAM-1) might block attachment of rhinoviruses. Pleconaril, a capsid inhibitor that integrates into the viral capsid in the VP1 hydrophobic pocket of the virus, is another agent under study. This can block capsid attachment to cells and perhaps also affect viral uncoating after entry. In vitro, pleconaril shows broad activity against picornaviruses, including enteroviruses. It remains to be seen whether these observations can be translated into effective preventive or therapeutic applications. At present, the attitude toward these viruses is best summed up by Sir Christopher Andrewes, who suggested that we should accept these infections as "one of the stimulating risks of being mortal."

## CORONAVIRUSES

Coronaviruses contain a single-stranded, positive-sense RNA genome, which is surrounded by an envelope that includes a lipid bilayer derived from intracellular rough endoplasmic reticulum and Golgi membranes of infected cells. Petal- or club-shaped spikes (peplomers) measuring approximately 13 nm project from the surface of the envelope, giving the appearance of a crown of thorns or a solar corona. The peplomers play an important role in inducing neutralizing and cellular immune responses. Like the rhinoviruses, coronaviruses are considered primary causes of the common cold. Based on serologic studies, it is estimated that they may cause as many as 5 to 10% of common colds in adults and a similar proportion of lower respiratory illnesses in children.

Enveloped RNA viruses

Disease similar to rhinoviruses

The number of serotypes is unknown. Two strains (229E and OC43) have been studied to some extent; it is clear that they can cause outbreaks similar to those of the rhinoviruses and that reinfection with the same serotype can occur. The cellular receptors for these strains are a cell surface metalloprotease and a sialic acid receptor similar to that bound by influenza C virus.

Metalloprotease and sialic acid receptors bind some strains

In late 2002, an illness called severe acute respiratory syndrome (SARS) appeared in China, spread throughout Asia, and is now found worldwide. The etiology has been identified as a previously undescribed coronavirus, with unusually high virulence for humans.

SARS is caused by a novel, new coronavirus

## REOVIRUSES

The reoviruses (respiratory enteric orphans) are naked virions that contain segmented, double-stranded RNA and replicate in the cytoplasm of infected cells. They are ubiquitous and have been found in humans, simians, rodents, cattle, and a variety of other hosts. They have been studied in great detail as experimental models, revealing much basic knowledge about viral genetics and pathogenesis at the molecular level. Three serotypes are known to infect humans; however, their role and importance in human disease remain uncertain.

Association with human disease is uncertain

## ADDITIONAL READING

### *Influenza Viruses*

Cox NJ, Subbarao K. Influenza. *Lancet* 1999;354:1277–1282. An excellent explanatory review of influenza virology, epidemiology, and prevention.

Gubareva LV, Kaiser L, Hayden FG. Influenza virus neuraminidase inhibitors. *Lancet* 2000;355:827–835. An update of the field of antivirals for influenza viruses.

Hatta M, Gao P, Halfmann P, Kawaoka Y. Molecular basis for high virulence of Hong Kong $H_5N_1$ influenza A viruses. *Science* 2001;293:1840–1842. This report, along with the accompanying article and editorial, further clarifies the molecular reasons for development of "novel," dangerous influenza viruses.

Neuzil KM, Zhu Y, Griffin MR, et al. Burden of interpandemic influenza in children younger than 5 years: A 25-year prospective study. *J Infect Dis* 2002;185:147–152. Healthy young children are usually not routinely immunized against influenza. This report details the frequency and types of morbidity that can occur, suggesting a reevaluation of this general policy.

Subbarao K, Klimov A, Katz J, et al. Characterization of an avian influenza A ($H_5N_1$) virus isolated from a child with a fatal respiratory illness. *Science* 1998;279:393–396. This article helps explain how an influenza virus might cross species barriers with serious consequences.

Taubenberger JK, Reid AH, Krafft AE, et al. Initial genetic characterization of the 1918 "Spanish" influenza virus. *Science* 1997;275:1793–1976. Using a preserved lung tissue sample from a victim of the 1918 pandemic, the investigators were able to detect viral RNA sequences that indicate the virus belonged to subgroup of strains that infect humans and pigs.

## Respiratory Syncytial Virus and Parainfluenza Viruses

Hall CB. Respiratory syncytial virus and parainfluenza virus. *N Engl J Med* 2001;344:1917–1928. This outstanding review further elucidates the nature of these viruses, their behavior, and what is currently known about their control.

Waris ME, Tsou C, Erdman DD, et al. Respiratory syncytial virus infection in BALB/c mice previously immunized with formalin-inactivated virus induces enhanced pulmonary inflammatory response with a predominant $Th_2$-like cytokine pattern. *J Virol* 1996;70:2852–2860. This study provides insight into the immunopathogenesis of respiratory syncytial virus and how vaccine-induced immunity can sometimes backfire.

## Adenoviruses

Bergelson JM, Cunningham JA, Droguett G, et al. Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. *Science* 1997;275:1320–1323. This article not only addresses fundamental questions about virus-cell interactions but is also relevant to the potential use of adenovirus vectors for gene therapy.

Tollefson AE, Scaria A, Hermiston TW, et al. The adenovirus death protein (E3-11.6K) is required at very late stages of infection for efficient cell lysis and release of adenovirus from infected cells. *J Virol* 1996;70:2296–2306. This and the next paper below illustrate the extraordinary ways in which adenoviruses can assure their ability to thrive and survive.

## Rhinoviruses

Marlin SD, Staunton DE, Springer TA, et al. A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection. *Nature* 1990;344:70–72. The experimental approach to defining the nature of a receptor and potential therapeutic applications is well illustrated.

## Coronaviruses

Myint SH. Human coronaviruses: A brief review. *Med Virol* 1994;4:35–46. An apt review of the history of coronavirus discovery, our evolving knowledge and what remains to be learned.

Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003; 348:1947–1958. This, and accompanying articles in the same issue, are remarkable for the spread with which a previously unknown, highly virulent agent has become characterized clinically, epidemiologically, and biologically.

## Reoviruses

Sharpe AH, Fields BN. Pathogenesis of viral infections: Basic concepts derived from the reovirus model. *N Engl J Med* 1985;312:486–497. A clearly presented review of the molecular basis of reovirus pathogenesis, with concepts that are relevant to our understanding of other viruses.

CHAPTER 34

# Mumps Virus, Measles, Rubella, and Other Childhood Exanthems

C. George Ray

The major viruses to be described in this chapter (mumps, measles, rubella, and the human parvovirus B19) are genetically unrelated; however, they share several common epidemiologic characteristics: (1) distribution is worldwide, with a high incidence of infection in nonimmune individuals; (2) humans appear to be the sole reservoir of infection; and (3) person-to-person spread is primarily by the respiratory (aerosol) route.

The other disease discussed in this chapter, roseola infantum, is a common illness in early life.

## Mumps

### Virology

Mumps virus is a paramyxovirus, and only one antigenic type is known. Like fellow members of its genus, it contains single-stranded, negative-sense RNA surrounded by an envelope. There are two glycoproteins on the surface of the envelope; one mediates neuraminidase and hemagglutination activity, and the other is responsible for lipid membrane fusion to the host cell.

Enveloped single-stranded RNA virus with hemagglutinating and neuraminidase activity

### Mumps Infection

Clinical Capsule

Before an effective vaccine against mumps was developed, the disease was a common childhood illness, commonly expressed as parotitis. It is also capable of causing aseptic meningitis, encephalitis, and (in adults) acute orchitis.

## EPIDEMIOLOGY

High infectivity is present before and after onset of illness

The highest frequency of mumps infection is observed in the 5- to 15-year age group. Infection is rarely seen in the first year of life. Although about 85% of susceptible household contacts acquire infection, approximately 30 to 40% of these contacts do not develop clinical disease. The disease is communicable from approximately 7 days before until 9 days after onset of illness; however, virus has been recovered in urine for up to 14 days following onset. The highest incidence of infection is usually during the late winter and spring months, but it can occur during any season.

## PATHOGENESIS

Viremic phase follows local replication

Viruria is common

After initial entry into the respiratory tract, the virus replicates locally. Replication is followed by viremic dissemination to target tissues such as the salivary glands and central nervous system (CNS). It is also possible that before development of immune responses, a secondary phase of viremia may result from virus replication in target tissues (eg, initial parotid involvement with later spread to other organs). Viruria is common, probably as a result of direct spread from the blood into the urine, as well as active viral replication in the kidney. The tissue response is that of cell necrosis and inflammation, with predominantly mononuclear cell infiltration. In the salivary glands, swelling and desquamation of necrotic epithelial lining cells, accompanied by interstitial inflammation and edema, may be seen within dilated ducts.

## IMMUNITY

Neutralizing antibody is protective

As in most viral infections, the early antibody response is predominantly with IgM, which is replaced gradually over several weeks by specific IgG antibody. The latter persists for a lifetime but can often be detected only by specific neutralization assays. Immunity is associated with the presence of neutralizing antibody. The role of cellular immune responses is not clear, but they may contribute both to the pathogenesis of the acute disease and to recovery from infection. After primary infection, immunity to reinfection is virtually always permanent.

# MUMPS: CLINICAL ASPECTS

## MANIFESTATIONS

Incubation period is 12–29 days

Parotitis is unilateral or bilateral

After an incubation period of 12 to 29 days (average, 16 to 18 days), the typical case is characterized by fever and swelling with tenderness of the salivary glands, especially the parotid glands. Swelling may be unilateral or bilateral and persists for 7 to 10 days. Several complications can occur, usually within 1 to 3 weeks of onset of illness. All appear to be a direct result of virus spread to other sites and illustrate the extensive tissue tropism of mumps.

Complications, which can occur without parotitis, include infection of the following:

1. Meninges: Approximately 10% of all infected patients develop meningitis. It is usually mild, but can be confused with bacterial meningitis. In about one third of these cases, associated or preceding evidence of parotitis is absent.
2. Brain: Encephalitis is occasionally severe.
3. Spinal cord and peripheral nerves: Transverse myelitis and polyneuritis are rare.
4. Pancreas: Pancreatitis is suggested by abdominal pain and vomiting.
5. Testes: Orchitis is estimated to occur in 10 to 20% of infected men. Although subsequent sterility is a concern, it appears that this outcome is quite rare.
6. Ovaries: Oophoritis is an unusual, usually benign inflammation of the ovarian glands.

Other rare and transient complications include myocarditis, nephritis, arthritis, thyroiditis, thrombocytopenic purpura, mastitis, and pneumonia. Most complications usually resolve without sequelae within 2 to 3 weeks. However, occasional permanent effects have been noted, particularly in cases of severe CNS infection, in which sensorineural hearing loss and other impairment can occur.

## DIAGNOSIS

Mumps virus can be readily isolated early in the illness from the saliva, pharynx, and other affected sites, such as the cerebrospinal fluid (CSF). The urine is also an excellent source for virus isolation. Mumps virus grows well in primary monolayer cell cultures derived from monkey kidney, producing syncytial giant cells and viral hemagglutinin. Rapid diagnosis can be made by direct detection of viral antigen in pharyngeal cells or urine sediment.

Cell culture of saliva, throat, CSF, and urine

The usual serologic tests are enzyme immunoassay (EIA) and indirect immunofluorescence to detect IgM- and IgG-specific antibody responses. Other serologic tests are also available, such as complement fixation, hemagglutination inhibition, and neutralization. Of these, the neutralization test is the most sensitive for detection of immunity to infection.

Viral antigen detected by immunofluorescence and EIA

EIA serology detects IgM and IgG

## PREVENTION

No specific therapy is available. Since 1967, a live attenuated vaccine that is safe and highly effective has been available. As a result of its routine use, infections in the United States are now exceedingly rare. The vaccine is produced by serial propagation of virus in chick embryo cell cultures. It is commonly combined with measles and rubella vaccines (MMR) and given as a single injection at 12 to 15 months of age. A second dose of MMR is recommended at 4 to 6 years of age; those who have missed the second dose should receive it no later than 11 to 12 years of age. A single dose causes seroconversion in more than 95% of recipients. Duration of immunity, especially if the two-dose regimen is followed, appears to be more than 25 years and may be lifelong.

Live vaccine given at 12–15 months of age

# MEASLES

## VIROLOGY

The measles virus is classified in the paramyxovirus family, genus *Morbillivirus.* It contains linear, negative-sense, single-stranded RNA, which encodes at least six virion structural proteins. Of these, three are in the envelope, comprising a matrix (M) protein that plays a key role in viral assembly and two types of glycoprotein projections (peplomers). One of the projections is a hemagglutinin (H), which mediates adsorption to cell surfaces; the other (F) mediates cell fusion, hemolysis, and viral entry into the cell. No neuraminidase activity is present. The receptor for measles virus is CD46 (membrane cofactor protein), a regulator of complement activation. Only a single serotype restricted to human infection is recognized; however, subtle antigenic and genetic variations among wild type measles strains do occur. These variations can be determined by sequencing analyses, enabling more precise epidemiologic tracking of outbreaks and their origins. Such ongoing molecular surveillance is also extremely important in determining whether significant antigenic drifts evolve over time.

Enveloped single-stranded RNA virus has hemagglutinating and fusion glycoproteins

CD46 is cell receptor

## MEASLES INFECTION

CLINICAL CAPSULE

Measles infections often produce severe illness in children, associated with high fever, widespread rash, and transient immunosuppression. This condition remains a major cause of mortality among children in developing countries.

## EPIDEMIOLOGY

Although a childhood disease, infections in young adults is important in transmission

Dramatic (99%) decrease recently in United States

Epidemics occur in unimmunized groups

The highest attack rates have been in children, usually sparing infants less than 6 months of age because of passively acquired antibody; however, a shift in age-specific attack rates to greater involvement of adolescents and young adults was observed in the United States in the 1980s. A marked decline in measles in the United States during the early 1990s may reflect decreased transmission as increased immunization coverage takes effect. However, in developing countries an estimated 1 million children still die from this disease each year.

Epidemics tend to occur during the winter and spring and increasingly are limited to one dose vaccine failures or groups who do not accept immunizations. The infection rate among exposed susceptible subjects in a classroom or household setting is estimated at 85%, and more than 95% of those infected become ill. The period of communicability is estimated to be 3 to 5 days before appearance of the rash to 4 days afterward.

## PATHOGENESIS

Respiratory cell multiplication disrupts cytoskeleton

Viremia disseminates to multiple sites

After implantation in the upper respiratory tract, viral replication proceeds in the respiratory mucosal epithelium. The effect within individual respiratory cells is profound. Even though measles does not directly restrict host cell metabolism, susceptible cells are damaged or destroyed by virtue of the intense viral replicative activity and the promotion of cell fusion with formation of syncytia. This results in disruption of the cellular cytoskeleton, chromosomal disorganization, and the appearance of inclusion bodies within the nucleus and cytoplasm. Replication is followed by viremic and lymphatic dissemination throughout the host to distant sites, including lymphoid tissues, bone marrow, abdominal viscera, and skin. Virus can be demonstrated in the blood during the first week after illness onset, and viruria persists for up to 4 days after the appearance of rash.

T and B lymphocytes are infected

Leukocyte function is impaired

Susceptibility to bacterial superinfections enhanced

During the viremic phase, measles virus infects T and B lymphocytes, circulating monocytes, and polymorphonuclear leukocytes without producing cytolysis. Profound depression of cell-mediated immunity occurs during the acute phase of illness and persists for several weeks thereafter. This is believed to be a result of virus-induced downregulation of interleukin-12 production by monocytes and macrophages. The effect on B lymphocytes has been shown to suppress immunoglobulin synthesis; in addition, generation of natural killer cell activity appears to be impaired. There is also evidence that the capability of polymorphonuclear leukocytes to generate oxygen radicals is diminished, perhaps directly by the virus or by activated suppressor T cells. This may further explain the enhanced susceptibility to bacterial superinfections. Virion components can be detected in biopsy specimens of Koplik's spots and vascular endothelial cells in the areas of skin rash.

Vasculitis, giant cells, and inclusions are seen

In addition to necrosis and inflammatory changes in the respiratory tract epithelium, several other features of measles virus infection are noteworthy. The skin lesions show vasculitis characterized by vascular dilation, edema, and perivascular mononuclear cell infiltrates. The lymphoid tissues show hyperplastic changes, and large multinucleated reticuloendothelial giant cells are often observed (Warthin–Finkeldey cells). Some of the giant cells contain intracytoplasmic and intranuclear inclusions. Similarly involved giant epithelial cells can be found in a variety of mucosal sites, the respiratory tract, skin, and urinary sediment.

Encephalitis lesions due to cytotoxic T-cell activity

The major findings in measles encephalitis include areas of edema, scattered petechial hemorrhages, perivascular mononuclear cell infiltrates, and necrosis of neurons. In most cases, perivenous demyelination in the CNS is also observed. The pathogenesis is thought to be related to infiltration by cytotoxic (CD8+) T cells, which react with myelin-forming or virus-infected brain cells.

## IMMUNITY

Lifelong immunity associated with neutralizing antibody

Cell-mediated immune responses to other antigens may be acutely depressed during measles infection and persist for several months. There is evidence that measles virus-specific cell-mediated immunity developing early in infection plays a role in mediating some of the features of disease, such as the rash, and is necessary to promote recovery from the illness.

Antibodies to the virus appear in the first few days of illness, peak in 2 to 3 weeks, and then persist at low levels. Immunity to reinfection is lifelong and is associated with the presence of neutralizing antibody. In patients with defects in cell-mediated immunity, including those with severe protein–calorie malnutrition, infection is prolonged, tissue involvement is more severe, and complications such as progressive viral pneumonia are common.

# MEASLES: CLINICAL ASPECTS

## MANIFESTATIONS

Common synonyms for measles include **rubeola,** 5-day measles, and hard measles. The incubation period ranges from 7 to 18 days. A typical illness usually begins 9 to 11 days after exposure, with cough, coryza, conjunctivitis, and fever. One to three days after onset, pinpoint gray–white spots surrounded by erythema (grains-of-salt appearance) appear on mucous membranes. This sign, called **Koplik's spots,** is usually most noticeable over the buccal mucosa opposite the molar teeth and persists for 1 to 2 days. Within a day of the appearance of Koplik's spots, the typical measles rash begins, first on the head, then on the trunk and extremities. The rash is maculopapular and semiconfluent; it persists for 3 to 5 days before fading. Fever and severe systemic symptoms gradually diminish as the rash progresses to the extremities. Lymphadenopathy is also common, with particularly noticeable involvement of the cervical nodes.

Incubation period is 7–18 days

Koplik's spots appear on mucous membranes

Rash spreads from head to trunk and extremities

Measles can be very severe, especially in immunocompromised or malnourished patients. Death can result from overwhelming viral infection of the host, with extensive involvement of the respiratory tract and other viscera. In some developing countries, mortality rates of 15 to 25% have been recorded.

### Complications

Bacterial superinfection, the most common complication, occurs in 5 to 15% of all cases. Such infections include acute otitis media, mastoiditis, sinusitis, pneumonia, and sepsis. Clinical signs of encephalitis develop in 1 of 500 to 1000 cases. This condition usually occurs 3 to 14 days after onset of illness and can be extremely severe. The mortality in measles encephalitis is approximately 15%, and permanent neurologic damage among survivors is estimated at 25%. Acute thrombocytopenic purpura may also develop during the acute phase of measles, leading to bleeding episodes. Abdominal pain and acute appendicitis can occur secondary to inflammation and swelling of lymphoid tissue.

Bacterial superinfection is common

Encephalitis can be severe

Thrombocytopenic purpura and bleeding occur in acute phase

### Subacute Sclerosing Panencephalitis

Subacute sclerosing panencephalitis is a rare, progressive neurologic disease of children, which usually begins 2 to 10 years after a measles infection. It is characterized by insidious onset of personality change, poor school performance, progressive intellectual deterioration, development of myoclonic jerks (periodic muscle spasms), and motor dysfunctions such as spasticity, tremors, loss of coordination, and ocular abnormalities, including blindness. Neurologic and intellectual deterioration generally progress over 6 to 12 months, with children eventually becoming bedridden and stuporous. Dysfunctions of the autonomic nervous system, such as difficulty with temperature regulation, may develop. Progressive inanition, superinfection, and metabolic imbalances eventually lead to death. Most of the pathologic features of the disease are localized to the CNS and retina. Both the gray and the white matter of the brain are involved, the most noteworthy feature being the presence of intranuclear and intracytoplasmic inclusions in oligodendroglial and neuronal cells.

Neurologic deterioration is progressive in children

Inclusions seen in neuronal cells

The disease is a result of chronic wild measles virus infection of the CNS. Studies have shown that patients have a variety of patterns of missing measles virus structural proteins in brain tissue. Thus, any of several defects in viral gene expression may prevent normal viral assembly, allowing persistence of defective virus at an intracellular site with failure of immune eradication.

Chronic measles virus infection

Incomplete measles virus is present in brain tissue

Rarely, a similar progressive, degenerative neurologic disorder may be related to persistent rubella virus infection of the CNS. This condition is seen most often in adolescents who have had congenital rubella syndrome. Rubella virus has been isolated from brain tissue in these patients, again using cocultivation techniques.

Incidence declined after introduction of measles vaccine

The incidence of subacute sclerosing panencephalitis is approximately one per 100,000 measles cases. Its occurrence in the United States has decreased markedly over the past 25 years with the widespread use of live measles vaccine. At present, there is no accepted effective therapy for subacute sclerosing panencephalitis.

## DIAGNOSIS

Rapid diagnosis is possible by immunofluorescence

The typical measles infection can often be diagnosed on the basis of clinical findings, but laboratory confirmation is necessary. Virus isolation from the oropharynx or urine is usually most productive in the first 5 days of illness. Measles grows on a variety of cell cultures, producing multinucleated giant cells similar to those observed in infected host tissues. If rapid diagnosis is desired, measles antigen may be identified in urinary sediment or pharyngeal cells by direct fluorescent antibody methods. Serologic diagnosis may involve complement fixation, hemagglutination inhibition, EIA, or indirect fluorescent antibody methods.

## TREATMENT

No specific therapy is available other than supportive measures and close observation for the development of complications such as bacterial superinfection. Intravenous ribavirin has been suggested for patients with severe measles pneumonia, but no controlled studies have been performed.

## PREVENTION

Live, attenuated vaccine is highly immunogenic

Vaccination is contraindicated in pregnant and immunocompromised individuals

Passive protection is appropriate for immunocompromised

Live, attenuated measles vaccine is available and highly immunogenic, most commonly administered as MMR. To ensure effective immunization, the vaccine should be administered to infants at 12 to 15 months of age with a second dose at 4 to 6 or 11 to 12 years of age. Immunity induced by the vaccine may be lifelong. Because the vaccine consists of live virus, it should not be administered to immunocompromised patients and it is not recommended for pregnant women. Exceptions to these guidelines include susceptible human immunodeficiency virus–infected persons. Exposed susceptible patients who are immunologically compromised (including small infants) may be given immune serum globulin intramuscularly. This treatment can modify or prevent disease if given within 6 days of exposure, but protection is transient.

# RUBELLA

Rubella was considered a mild, benign exanthem of childhood until 1941, when the Australian ophthalmologist Sir Norman Gregg described the profound defects that could be induced in the fetus as a result of maternal infection. Since 1962, when the virus was first isolated, knowledge regarding its extreme medical importance and biological characteristics has increased rapidly.

## VIROLOGY

Enveloped togavirus contains single-stranded RNA

Rubella virus is classified as a member of the togavirus family. It is enveloped and contains single-stranded, positive-sense RNA. There is only one serotype, and no extrahuman reservoirs are known to exist. The virus can agglutinate some types of red blood cells, such as those obtained from 1-day-old chicks and trypsin-treated human type O cells.

# RUBELLA INFECTION

CLINICAL CAPSULE

Infections by rubella virus are often mild, or even asymptomatic. The major concerns are the profound effects on developing fetuses, resulting in multiple congenital malformations.

## EPIDEMIOLOGY

Infections are usually observed during the winter and spring months. In contrast to measles, which has a high clinical attack rate among exposed susceptible individuals, only 30 to 60% of rubella-infected susceptible persons develop clinically apparent disease. A major focus of concern is susceptible women of childbearing age, who carry a risk of exposure during pregnancy. Patients with primary acquired infections are contagious from 7 days before to 7 days after the onset of rash; congenitally infected infants may spread the virus to others for 6 months or longer after birth.

Virus has high infectivity but low virulence

Childbearing women are the major concern

## PATHOGENESIS

In acquired infection, the virus enters the host through the upper respiratory tract, replicates, and then spreads by the bloodstream to distant sites, including lymphoid tissues, skin, and organs. Viremia in these infections has been detected for as long as 8 days before to 2 days after onset of the rash, and virus shedding from the oropharynx can be detected up to 8 days after onset (Fig 34–1). Cellular immune responses and circulating virus–antibody immune complexes are thought to play a role in mediating the inflammatory responses to infection, such as rash and arthritis.

Cellular immune responses and virus–antibody complexes mediate arthritis and rash

Congenital infection occurs as a result of maternal viremia that leads to placental infection and then transplacental spread to the fetus. Once fetal infection occurs, it persists chronically. Such persistence is probably related to an inability to eliminate the virus by immune or interferon-mediated mechanisms. There is too little inflammatory change in the fetal tissues to explain the pathogenesis of the congenital defects. Possibilities include placental and fetal vasculitis with compromise of fetal oxygenation, chronic viral infection of cells leading to impaired mitosis, cellular necrosis, and induction of chromosomal breakage. Any or all of these factors may operate at a critical stage of organogenesis to induce permanent defects. Viral persistence with circulating virus–antibody immune

Transmission to fetus by viremia

Fetal infection becomes chronic

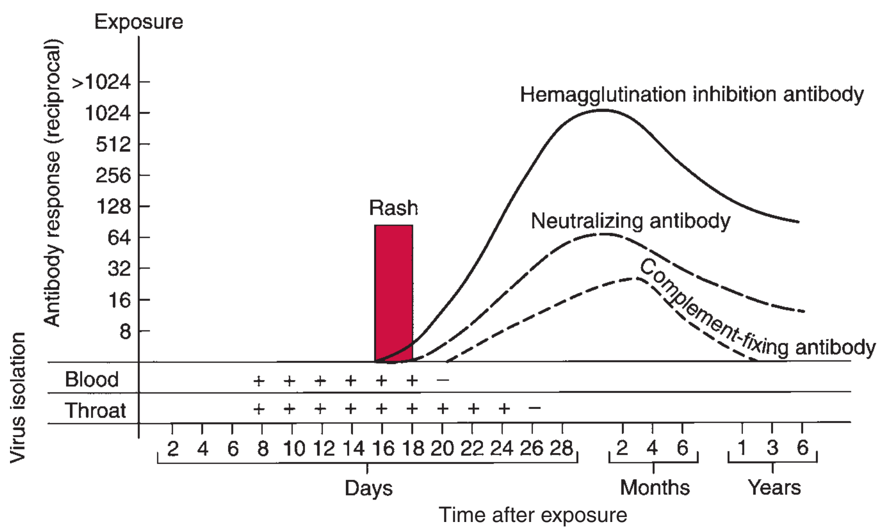


FIGURE 34–1
Antibody response and viral isolation in a typical case of acquired rubella.

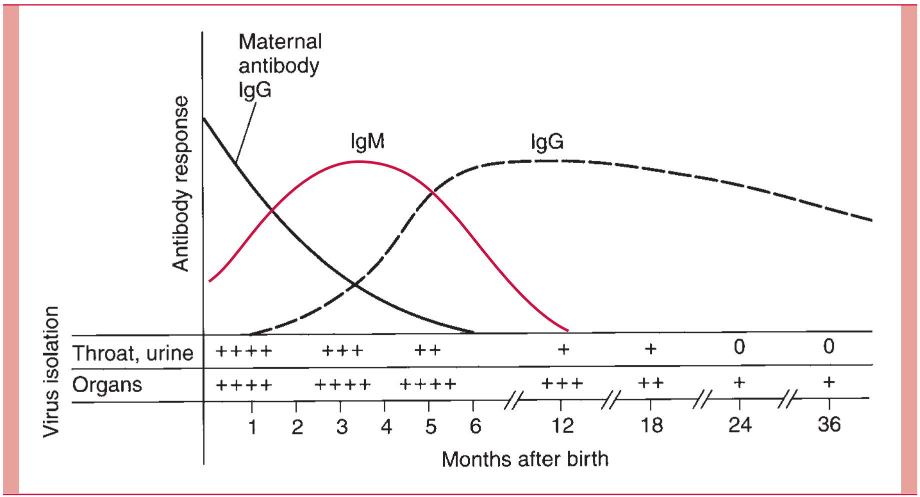


FIGURE 34–2
Persistence of rubella virus and antibody in congenitally infected infants.

complexes may evoke inflammatory changes postnatally and produce continuing tissue damage.

Infection and virus shedding continue long after birth

Virus persists despite antibody

After birth, affected infants continue to excrete the virus in the throat, urine, and intestinal tract (Fig 34–2). Virus may be isolated from virtually all tissues in the first few weeks of life. Shedding of virus in the throat and urine, which persists for at least 6 months in most cases, has been known to continue for 30 months. Virus has also been isolated from lens tissue removed 3 to 4 years later. These observations underscore the fact that such infants are important reservoirs in perpetuating virus transmission. The prolonged virus shedding is somewhat puzzling; it does not represent a typical example of immunologic tolerance. The affected infants are usually able to produce circulating IgM and IgG antibodies to the virus (see Fig 34–2), although antibodies may decrease to undetectable levels after 3 to 4 years. Many infants show evidence of depressed rubella virus-specific cell-mediated immunity during the first year of life.

## PATHOLOGY

Fetal disease includes multiple malformations

Because postnatally acquired disease is usually mild, little is known about its pathology. Mononuclear cell inflammatory changes can be observed in tissues, and viral antigen can be detected in the same sites (eg, skin and synovial fluid). Congenital infections are characterized primarily by the various malformations. Necrosis of tissues such as myocardium and vascular endothelium may also be seen, and quantitative studies suggest a decrease in cell quantity in affected organs. In severe cases, normal calcium deposition in the metaphyses of long bones is delayed, sometimes referred to as a “celery stalk” appearance on a radiograph.

## IMMUNITY

Lasting immunity is associated with IgG and IgA

After infection the serum antibody titer rises, reaching a peak within 2 to 3 weeks of onset. Natural infection also results in the production of specific secretory IgA antibodies in the respiratory tract. Immunity to disease is nearly always lifelong; however, reexposure can lead to transient respiratory tract infection, with an anamnestic rise in IgG and secretory IgA antibodies, but without resultant viremia or illness.

# RUBELLA: CLINICAL ASPECTS

## MANIFESTATIONS

Rubella is commonly known as **German measles** or 3-day measles. The incubation period for acquired infection is 14 to 21 days (average, 16 days). Illness is generally very mild, consisting primarily of low-grade fever, upper respiratory symptoms, and lymphadenopathy, which is most prominent in the posterior cervical and postauricular areas. A macular rash often follows within a day of onset and lasts 1 to 3 days. This rash, which is often quite faint, is usually most prominent over the head, neck, and trunk. Petechial lesions may also be seen over the soft palate during the acute phase. The most common complication is arthralgia or overt arthritis, which may affect the joints of the fingers, wrists, elbows, knees, and ankles. The joint problems, which occur most frequently in women, rarely last longer than a few days to 3 weeks. Other, rarer complications include thrombocytopenic purpura and encephalitis.

Illness is mild with lymphadenopathy and macular rash

Arthralgia and arthritis is common in women

The major significance of rubella is not the acute illness but the risk of fetal damage in pregnant women, particularly when they contract either symptomatic or subclinical primary infection during the first trimester. The risk of fetal malformation and chronic fetal infection, which is estimated to be as high as 80% if infection occurs in the first 2 weeks of gestation, decreases to 6 to 10% by the 14th week. The overall risk during the first trimester is estimated at 20 to 30%.

Clinical manifestations of congenital rubella syndrome vary, but may include any combination of the following major findings: cardiac defects, commonly patent ductus arteriosus and pulmonary valvular stenosis; eye defects such as cataracts, chorioretinitis, glaucoma, coloboma, cloudy cornea, and microphthalmia; sensorineural deafness; enlargement of liver and spleen; thrombocytopenia; and intrauterine growth restriction. Other findings include CNS defects such as microcephaly, mental retardation, and encephalitis; anemia; transient immunodeficiency; interstitial pneumonia; and intravascular coagulation; hepatitis; rash; and other congenital malformations. Late complications of congenital rubella syndrome have also been described, including an increased risk of diabetes mellitus, chronic thyroiditis, and occasionally the development of a progressive subacute panencephalitis in the second decade of life. Some congenitally infected infants may appear entirely normal at birth, and sequelae such as hearing or learning deficits may not become apparent until months later. The spectrum of defects thus varies from subtle to severe.

High risk for fetal damage with infection in first trimester

Lesions of congenital rubella include multiple body systems

## DIAGNOSIS

Because of the rather nonspecific nature of the illness, a diagnosis of rubella cannot be made on clinical grounds alone. More than 30 other viral agents, which are discussed later in this chapter, can produce a similar illness. Confirmation of the diagnosis requires laboratory studies. The virus may be isolated from respiratory secretions in the acute phase (and from urine, tissues, and feces in congenitally infected infants) by inoculation into a variety of cell cultures, or detected by reverse transcriptase polymerase chain reaction. Serologic diagnosis is most commonly used in acquired infections; paired acute and convalescent samples collected 10 to 21 days apart are used. Hemagglutination inhibition, indirect immunofluorescence, EIA, and other tests are available.

Acquired infections are diagnosed serologically

Determination of IgM-specific antibody is sometimes useful to ascertain whether an infection occurred in the past several months; it has also been used in the diagnosis of congenital infections. Unfortunately, there are certain pitfalls in interpreting this test. Some individuals (<5%) with acquired infections may have persistent elevations of IgM-specific antibodies for 200 days or more afterward, and some congenitally infected infants do not produce detectable IgM-specific antibodies.

IgM tests can help detect congenital infections

## TREATMENT AND PREVENTION

Other than supportive measures, there is no specific therapy for either the acquired or the congenital infection.

Since 1969, a live attenuated rubella vaccine has been available for routine immunization. As a result of the widespread use of the vaccine in the United States, the number of cases of rubella has declined dramatically. From 1990 through 1999, the median number of cases reported annually was only 232. The current vaccine virus, grown in human diploid fibroblast cell cultures (RA 27/3), has been shown to be highly effective.

Live attenuated rubella vaccine is indicated for children and hospital workers

It causes seroconversion in approximately 95% of recipients. Routine immunization is now recommended for infants after the first year of life and for other individuals with no history of immunization and lack of immunity by serologic testing. Target groups include female adolescents and hospital personnel in high-risk settings. The vaccine is contraindicated in many immunocompromised patients and in pregnancy. To date, more than 200 instances of accidental vaccination of susceptible pregnant women have been reported, with no clinically apparent adverse effects on the fetus; however, it is strongly recommended that immunization be avoided in this setting and that nonpregnant women avoid conception for at least 3 months after receiving the vaccine. Vaccine-induced immunity may be lifelong. Studies to date indicate that the duration of protection is at least 16 years.

Vaccine does not produce defects in fetus

Vaccine-induced immunity may be lifelong

## PARVOVIRUS B19 INFECTIONS

Parvoviruses are very small (18 to 26 nm), naked virions that contain a linear single-stranded DNA molecule. Diseases caused by parvoviruses have been recognized among nonhuman hosts for a number of years. Notable among these are canine parvovirus and feline panleukopenia virus, which produce particularly severe infections among puppies and kittens, respectively. These do not appear to cross species barriers. The human parvovirus B19 has been well described, but its origin is not yet known.

Small naked, single-stranded DNA viruses

Parvovirus B19 encodes three capsid proteins (VP1, VP2, and VP3). The virus can be grown in primary cultures of human bone marrow cells, fetal liver cells, hematopoietic progenitor cells generated from peripheral blood, and a megakaryocytic leukemia cell line. The major cellular receptor for the virus is globoside (also known as blood group P antigen, which is commonly found on erythroid progenitors, erythroblasts, megakaryocytes, and endothelial cells). All represent potential targets for disease production. A primary site of replication appears to be the nucleus of an immature cell in the erythrocyte lineage. Such infected cells then cease to proliferate, resulting in an impairment of normal erythrocyte development.

The clinical consequences of this effect on erythrocytes are generally trivial, unless patients are already compromised by a chronic hemolytic process, such as sickle cell disease or thalassemia, in which maximal erythropoiesis is continually needed to counterbalance increased destruction of circulating erythrocytes. Primary infection by parvovirus B19 in such individuals often produces an acute, severe, sometimes fatal anemia manifested as a rapid fall in red blood cell counts and hemoglobin. Patients may present initially with no clinical symptoms other than fever, and is commonly referred to as **aplastic crisis.** Immunocompromised patients such as those with acquired immunodeficiency syndrome sometimes have difficulty clearing the virus and develop persistent anemia with reticulocytopenia. Parvovirus B19 has also been occasionally implicated as a cause of persistent bone marrow failure and an acute hemophagocytic syndrome.

Replicates in erythroid precursor nuclei

Globoside is virus receptor

Endothelial cells and megakaryocytes can also be affected

Aplastic crisis develops in patients with chronic hemolytic anemias

**Erythema infectiosum** (also referred to as fifth disease or academy rash) is a more common disease that is clearly attributable to parvovirus B19. After an incubation period of 4 to 12 days, a mild illness appears, characterized by fever, malaise, headache, myalgia, and itching in varying degrees. A confluent, indurated rash appears on the face, giving a “slapped-cheek” appearance. The rash spreads in a day or two to other areas, particularly exposed surfaces such as the arms and legs, where it is usually macular and reticular (lace-like). During the acute phase, generalized lymphadenopathy or splenomegaly may be seen, along with a mild leukopenia and anemia.

The illness lasts 1 to 2 weeks, but rash may recur for periods of 2 to 4 weeks thereafter, exacerbated by heat, sunlight, exercise, or emotional stress. Arthralgia sometimes persists or recurs for weeks to months, particularly in adolescent or adult females. Overt arthritis or vasculitis have also been reported in some individuals. Serious complications,

Erythema infectiosum is usually mild “slapped cheek” rash such as hepatitis, thrombocytopenia, nephritis or encephalitis are rare. However, like rubella, active transplacental transmission of parvovirus B19 can occur during primary infections in the first 20 weeks of pregnancy, sometimes resulting in stillbirth of fetuses that are profoundly anemic. The progress can be so severe that hypoxic damage to the heart, liver, and other tissues leads to extensive edema (hydrops fetalis). The frequency of such adverse outcomes is as yet undetermined.

Fetal infection is occasionally severe

Fetal anemia leads to hydrops fetalis

It is important to be aware that erythema infectiosum is extremely variable in its clinical manifestations; even the “classic” presentation can be mimicked by other agents, such as rubella and echoviruses. Before a firm diagnosis is made on clinical grounds, especially during outbreaks, it is wise to exclude the possibility of atypical rubella infection.

Epidemiologic evidence suggests that spread of the virus is primarily by the respiratory route, and high transmission rates occur in households. Outbreaks tend to be small and localized, particularly during the spring months, with the highest rates among children and young adults. Seroepidemiologic studies have demonstrated evidence of past infection in 30 to 60% of adults. Viremia usually lasts 7 to 12 days but can persist for months in some individuals. It can be detected by specific DNA probe or polymerase chain reaction (PCR) methods. Alternatively, the presence of IgM-specific antibody late in the acute phase or during convalescence strongly supports the diagnosis.

Detection requires DNA probe or PCR

IgM-specific antibody supports diagnosis

## Roseola Infantum (Exanthem Subitum)

Roseola infantum is a common illness observed in infants and children 6 months to 4 years of age. Its alternative name, exanthem subitum, means “sudden rash.” Roseola has more than one cause: the most common is human herpesvirus type 6 and, less frequently, human herpesvirus type 7 (see Chapter 38). Several other agents, including adenoviruses, coxsackieviruses, and echoviruses, have occasionally been noted to cause similar manifestations. The illness is characterized by abrupt onset of high fever, sometimes accompanied by brief, generalized convulsions and leukopenia. After 3 to 5 days, the fever diminishes rapidly, followed in a few hours by a faint, transient, macular rash.

Associated with human herpesvirus type 6 or type 7

## Other Causes of Rubella-like Rashes

In addition to erythema infectiosum, diseases caused by numerous other agents can mimic rubella. These include at least 17 echoviruses, 9 coxsackieviruses, several adenoviral serotypes, arboviruses such as dengue, Epstein–Barr virus, scarlet fever, and toxic drug eruptions. Because of the wide variety of diagnostic possibilities, it is not possible to diagnose or rule out rubella confidently on clinical grounds alone. Therefore, a specific diagnosis requires specific laboratory studies. Because rubella is an infection with such significant impact on the fetus, serologic study to rule out the possibility is mandatory if the diagnosis is suspected during early pregnancy.

## ADDITIONAL READING

### Mumps

Cheek JE, Baron R, Atlas H, et al. Mumps outbreak in a highly vaccinated school population. *Arch Pediatr Adolesc Med* 1995;149:774–778. An illustration of the importance of monitoring and implementing immunization programs.

## Measles

Atabani SF, Syrnes AA, Jay A, et al. Natural measles causes prolonged suppression of interleukin-12 production. *J Infect Dis* 2001;184:1–9. An article that provides insight into the immunopathogenesis of measles virus infections.

Rota JS, Rota PA, Redd SB, et al. Genetic analysis of measles viruses isolated in the United States, 1995–1996. *J Infect Dis* 1998;177:204–208. This article illustrates how molecular epidemiologic studies can be used to monitor virus spread in populations and determine antigenic drift.

## Rubella

Miller E, Cradock-Watson JE, Pollock TM. Consequences of confirmed maternal rubella at successive stages of pregnancy. *Lancet* 1982;2:781–784. A precise analysis of the risks of infection at various times during gestation.

Reef SE, Frey TK, Theall K, et al. The changing epidemiology of rubella in the 1990s. *JAMA* 2002;287:464–472. This report and the accompanying editorial highlight the profound impact resulting from widespread immunization and examine the barriers to ultimate eradication.

## Parvovirus B19

Harel L, Straussberg R, Rudich H, et al. Raynaud's phenomenon as a manifestation of parvovirus B19 infection: Case reports and review of parvovirus B19 rheumatic and vasculitic syndromes. *Clin Infect Dis* 2000;30:500–503. An article that provides further insight into pathogenesis.

Heegaard ED, Hornsleth A. Parvovirus: The expanding spectrum of disease. *Acta Paediatr* 1995;84:109–117. The history of parvovirus B19, along with the broad spectrum of disease expression, is reviewed.

CHAPTER 35

# Poxviruses

C. George Ray

The poxvirus family includes viruses that infect birds, mammals, and even insects. The agents most important in human disease are variola (smallpox), vaccinia, molluscum contagiosum, orf, cowpox, and pseudocowpox (Table 35–1).

## Poxviruses: Group Characteristics

Poxviruses are large, brick-shaped or ovoid, double-stranded DNA-carrying virions (Fig 35–1) measuring approximately 100 × 200 × 300 nm. Their structure is complex, and replication occurs in the cytoplasm of infected cells. They possess an envelope, which is not acquired by budding and not essential for infectivity.

Double-stranded DNA

Replication in cytoplasm

## Variola (Smallpox)

### Virology

Two virus types are known: variola major and variola minor (alastrim). Although the viruses are indistinguishable antigenically, their fatality rates differ considerably (<1% for variola minor, 3–35% for variola major). They are also difficult to distinguish in the laboratory; however, variola major has slightly greater virulence in embryonated hen's eggs.

Variola major and minor are difficult to distinguish

### Smallpox

Clinical Capsule

Smallpox is an acute infection in which the dominant feature is a uniform papulovesicular rash that evolves to pustules over 1 to 2 weeks. The potential for spread and mortality is significant, particularly in a nonimmune population.

TABLE 35–1

**Poxviridae that Affect Humans**

| GENERA | DISEASES |
|---|---|
| *Orthopoxvirus* | Variola<br>Vaccinia<br>Cowpox[a]<br>Monkeypox[a] |
| *Parapoxvirus* | Bovine papular stomatitis[a]<br>Orf[a]<br>Pseudocowpox[a] |
| *Molluscipoxvirus* | Molluscum contagiosum |
| *Yatapoxvirus* | Tanapox[a]<br>Yatapox[a] |

[a] Viruses that have nonhuman reservoirs but can cause disease in humans (usually mild and localized).

Person-to-person communicability by respiratory droplets and fomites is high

Smallpox has played a significant role in world history with respect to both the serious epidemics recorded since antiquity and the sometimes dangerous measures taken to prevent infection. Smallpox virus is highly contagious and can survive well in the extracellular environment. Acquisition of infection by infected saliva droplets or by exposure to skin lesions, contaminated articles, and fomites has been well documented.

WHO eradication campaign based on lack of nonhuman reservoir and asymptomatic cases

Immunization and case tracing led to success in 1980

In 1967, the World Health Organization (WHO) launched an ambitious program aimed at eradication of smallpox. This goal was considered realistic for two major reasons: (1) no extrahuman reservoir of the virus was known to exist, and (2) asymptomatic carriage apparently did not occur. The basic approach included intensive surveillance for clinical cases of smallpox, prompt quarantine of such patients and their contacts, and immunization of contacts with vaccinia virus (vaccination) to prevent further spread. A tremendous amount of effort was involved, but the results were astonishing: the last recorded case of naturally acquired smallpox occurred in Somalia in 1977. Global eradication of smallpox was confirmed in 1979 and accepted by the WHO in May 1980. Since then, the virus has been solely secured in two WHO-restricted laboratories: one at the United States Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, and the other at a similar facility in Moscow, Russia.

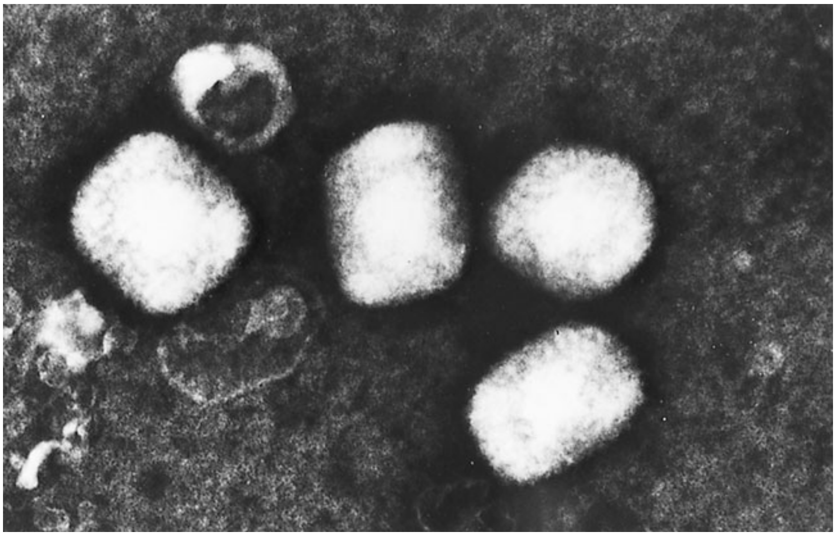

FIGURE 35–1
Electron microscopic appearance of a poxvirus (vaccinia). (Negative stain; original magnification × 60,000.) (*Courtesy of Dr. Claire M. Payne.*)

Unfortunately, the dramatic world events that occurred in 2001 have raised the chilling possibility that clandestine virus stocks may exist elsewhere and could be effectively used for major bioterrorist attacks. Reasons for such concern include (1) known high infectivity among humans; (2) high susceptibility among populations (routine vaccination against smallpox ended in 1972, and current vaccine supplies are limited); (3) risk that health care providers may not promptly recognize and respond to early cases; and (4) absence of specific antiviral treatment. A response plan and guidelines for such threats is posted on a CDC website **(www.cdc.gov/nip/smallpox)** and is updated at regular intervals.

Potential bioterrorist weapon

No proven antiviral treatment

Continuing surveillance also includes studies of poxviruses of animals (eg, buffalopox, monkeypox) that are antigenically somewhat similar to smallpox. Some virologists remain legitimately concerned that an animal poxvirus, such as monkeypox, could mutate to become highly virulent to humans—a further reminder that complacency could be dangerous.

Animal poxviruses could be a future threat

## PATHOGENESIS

The orthopoxviruses as a group cause a dramatic effect on host cell macromolecular function, leading to a switch from cellular to viral protein synthesis, changes in cell membrane permeability and cytolysis. Eosinophilic inclusions, called **Guarnieri's bodies,** can be seen in the cytoplasm. Multiple viral proteins, such as complement regulatory protein and other factors that can interfere with induction or activities of multiple host mononuclear cell cytokines, are also synthesized; this serves to impair the host defenses that are important in early control of infection.

Profound effect on host cell protein synthesis

Viral proteins undermine host defenses

# SMALLPOX: CLINICAL ASPECTS

## MANIFESTATIONS AND DIAGNOSIS

The incubation period of smallpox is usually 12 to 14 days, although in occasional fulminating cases it can be as short as 4 to 5 days. The typical onset is abrupt, with fever, chills, and myalgia, followed by a rash 3 to 4 days later. The rash evolves to firm papulovesicles that become pustular over 10 to 12 days, then crust and slowly heal. Only a single crop of lesions (all in the same stage of evolution) develop; these lesions are most prominent over the head and extremities (Fig 35–2). Some cases are fulminant, with a hemorrhagic rash ("sledgehammer" smallpox). Death can result from the overwhelming primary viral infection or from bacterial superinfection. Diagnostic methods utilize vesicular scrapings, and include culture, electron microscopy, gel diffusion, and polymerase chain reaction.

Single-stage rash

Vesicular scrapings used for diagnosis

## PREVENTION

The first major step toward modern prevention and subsequent eradication of smallpox can be credited to Edward Jenner, who noted that milkmaids who develop mild cowpox lesions on their hands appeared immune to smallpox. In 1798, he published evidence indicating that purposeful inoculation of individuals with cowpox material could protect them against subsequent infection by smallpox. The concept of vaccination gradually evolved, with the modern use of live vaccinia virus, a poxvirus of uncertain origin to be discussed later, which produced specific immunity.

Jenner vaccinated with cowpox

# VACCINIA

Vaccinia virus is serologically related to smallpox, although its exact origin is unknown. Some virologists believe it is a recombinant virus derived from smallpox and cowpox, and others suggest it originated from a poxvirus of horses. The virus is usually propagated by

Origin is unknown

FIGURE 35–2
Closeup of facial lesions of smallpox during the first week of the illness.

Vaccination produces strong local reactions

Severe reactions seen in immunocompromised

Immunity wanes after 3 years

dermal inoculation of calves, and the resultant vesicle fluid ("lymph") is lyophilized and used as a live virus vaccine in humans. The vaccine is inoculated into the epidermis and produces a localized lesion, which indicates successful immunization. The lesion becomes vesicular, then pustular, followed by crusting and healing over 10 to 14 days. The local reaction is sometimes severe and accompanied by systemic symptoms such as fever, rash, and lymphadenopathy. Patients who are immunocompromised may experience severe reactions, such as progressive vaccinia. Vaccinia-produced immunity to smallpox wanes rapidly after 3 years, and the duration of long-term immunity beyond that time is uncertain.

Vaccinia of interest as mechanism for delivering the immunogenic proteins of other viruses

There has been a resurgence of scientific interest in vaccinia as a possible vector for active immunization against other diseases, such as hepatitis B, herpes simplex, and even human immunodeficiency virus. It has been shown that gene sequences coding for specific immunogenic proteins of other viruses can be inserted into the vaccinia virus genome, with subsequent expression as the virus replicates. For example, a recombinant vaccinia strain carrying the gene sequence for hepatitis B surface antigen (HbsAg) can infect cells, lead to production of HbsAg, and stimulate an antibody response to it. Theoretically, gene sequences coding for a variety of antigens could be packaged in a single viable vaccinia virus, thus allowing simultaneous active immunization against multiple agents. It has been suggested that use of other poxviruses of animal or avian orgin, such as canarypox, may be even safer, yet effective vectors for use in humans. Whether such approaches become routinely applicable to clinical medicine remains to be seen.

## MOLLUSCUM CONTAGIOSUM

Transmission is direct skin-to-skin

Molluscum contagiosum is a benign, cutaneous poxvirus disease of humans, spread by direct contact with infected cells. It is usually acquired by inoculation into minute skin abrasions; events that commonly lead to transmission include "roughhousing" in shower rooms and swimming pools, sharing of towels, and sexual contact.

After an incubation period of 2 to 8 weeks, nodular, pale, firm (pearl-like) lesions usually 2 to 10 mm in diameter develop in the epidermis. These lesions are painless and umbilicated in appearance. A cheesy material may be expressed from the pore at the center of each lesion. Local trauma may cause spread of lesions in the involved skin area. The lesions are not associated with systemic symptoms, and they disappear in 2 to 12 months without treatment. Specific treatment, if desired, is usually by curettage or careful removal of the central core by expression with forceps.

Painless lesions express cheesy material

Pathologic findings, which are limited to the epidermis, include hyperplasia, ballooning degeneration, and acanthosis. The diagnosis, made on clinical grounds, can be confirmed by demonstration of large, eosinophilic cytoplasmic inclusions (molluscum bodies) in the affected superficial epithelial cells.

Molluscum bodies in cytoplasm are diagnostic

## ORF

**Orf** is an old Saxon term for a human infection caused by a parapoxvirus of sheep and goats. Synonyms for the infection in animals include contagious pustular dermatitis, ecthyma contagiosum, pustular ecthyma, and "scabby mouth." Humans usually acquire the infection by close contact with infected animals and accidental inoculation through cuts or abrasions on the hand or wrist. The typical skin lesion is solitary; it begins as a vesicle and then evolves into a nodular mass that later develops central necrosis. Regional lymphadenopathy sometimes develops. Dissemination is rare. The average duration of the lesion is 35 days, followed by complete resolution. The diagnosis is usually made on the basis of clinical appearance and occupational history. Serologic confirmation or electron microscopy of the lesion can be performed but is rarely necessary.

Vesicular skin lesions seen in sheep- or goat-herders

## MILKER'S NODULES AND COWPOX

Milker's nodules (pseudocowpox) is a cutaneous parapoxvirus disease of cattle, distinct from cowpox, that can cause local skin infections similar to orf in exposed humans. Healing of the skin lesions may take 4 to 8 weeks. There is no cross-immunity to cowpox. Cowpox is now very rare in the United States. It produces a vesicular eruption on the udders of cows and similar, usually localized, vesicular skin lesions in humans who are accidentally exposed.

Localized infection acquired by direct contact with bovines

## ADDITIONAL READING

Barquet N, Domingo P. Smallpox: The triumph over the most terrible of the ministers of death. *Ann Intern Med* 1997;127:635–642. This first-rate account of the history, science, and successful eradication of this agent is highly recommended.

Breman JG, Henderson DA: Diagnosis and management of smallpox. *N Engl J Med* 2002;346:1300–1308. This updated review and accompanying articles in the same issue highlight the resurgence in concern that smallpox may return as a threat to humanity.

Cadoz M, Strady A, Meignier B, et al. Immunization with canarypox virus expressing rabies glycoprotein. *Lancet* 1992;339:1429–1432. A nonhuman poxvirus that undergoes only abortive replication in mammalian cells is exploited as a potentially safe vector for immunization. The editorial on pages 1448–1449 is also worthwhile reading.

Cohen J. Is an old virus up to new tricks? *Science* 1997;277:312–313. This article is a vivid reminder that although one scourge may have been eradicated, others may be capable of taking its place.

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CHAPTER 36

# Enteroviruses

C. George Ray

Enteroviruses constitute a major subgroup of small RNA viruses (picornaviruses) that readily infect the intestinal tract. The enteroviruses of humans and animals are ubiquitous and have been found worldwide. Their name is derived from their ability to infect intestinal tract epithelial and lymphoid tissues and to be shed into the feces. They include the polioviruses, coxsackieviruses, echoviruses, and more recently discovered agents that are simply designated enteroviruses.

These viruses, which have many characteristics in common, are first considered as a group. Some of the special features of important serotypes will be discussed in more detail later in this chapter.

## *Enteroviruses: Group Characteristics*

### VIROLOGY

### MORPHOLOGY AND BIOLOGICAL FEATURES

As a group, the enteroviruses are extremely small (22 to 30 nm in diameter), naked virions with icosahedral symmetry. They possess single-stranded, positive-sense RNA and a capsid formed from 60 copies of four nonglycosylated proteins (VP1, VP2, VP3, VP4). Replication and assembly occurs exclusively in the cellular cytoplasm; one infectious cycle can occur within 6 to 7 hours. This results in cessation of host cell protein synthesis and cell lysis with release of new infectious progeny.

Small, single-stranded RNA viruses

Replication and assembly takes place in cytoplasm

Unlike rhinoviruses, which are also members of the picornavirus family, enteroviruses are quite resistant to an acid pH (as low as 3.0). This feature undoubtedly helps ensure their survival during passage through the stomach to the intestines. Enteroviruses are also resistant to many common disinfectants such as 70% alcohol, substituted phenolics, ether, and various detergents that readily inactivate most enveloped viruses. Chemical agents, such as 0.3% formaldehyde or free residual chlorine at 0.3 to 0.5 ppm, are effective; however, if sufficient extraneous organic debris is present, the virus can be protected and survive long periods.

Resistant to acid, detergents, and many disinfectants

Formaldehyde and hypochlorite are active against enteroviruses

Some of the enterovirus serotypes share common antigens, but there are no significant serologic relationships between the major classes listed in Table 36–1. Genetic variation within specific strains occurs, and mutants that exhibit antigenic drift and altered tropism for specific cell types are now recognized. Polioviruses, which have been most extensively

Antigenic mutations and drifts occur

TABLE 36–1

**Human Enterovirusesa**

| Class | Number of Serotypes[a] |
|---|---|
| Poliovirus | 3 |
| Coxsackievirus | |
| Group A | 23 |
| Group B | 6 |
| Echovirus | 28 |
| Enterovirus | 4 |

[a] More recently discovered enteroviruses, which have overlapping biological characteristics, are identified numerically (types 68–71). Two of the original 30 numbered echovirus serotypes have been reclassified; however, the remaining retain their original serotype number (eg, echovirus 30).

Antibody to surface proteins neutralize infectivity

studied as enterovirus prototypes, are known to have epitopes on three surface structural proteins (VP1, VP2, and VP3) that induce type-specific neutralizing antibodies. This appears to be generally the case for all enteroviruses; definitive identification of isolates usually requires neutralization tests.

## GROWTH IN THE LABORATORY

Growth of some in primate cell cultures

Coxsackie A and B viruses have different effects on newborn mice

Most enteroviruses can be isolated in primate (human or simian) cell cultures and show characteristic cytopathic effects. Some strains, particularly several coxsackievirus A serotypes, are more readily detected by inoculation of newborn mice. In fact, the newborn mouse is one basis for originally classifying group A and B coxsackieviruses. Group A coxsackieviruses cause primarily a widespread, inflammatory, necrotic effect on skeletal muscle, leading to flaccid paralysis and death. Similar inoculation of group B coxsackieviruses causes encephalitis, resulting in spasticity and occasionally convulsions. Echoviruses and polioviruses rarely have an adverse effect on mice, unless special adaptation procedures are first employed. The higher-numbered enteroviruses (types 68–71), which have overlapping, variable growth and host characteristics, have been classified separately.

# ENTEROVIRUS DISEASE

CLINICAL CAPSULE

Enterovirus infections can produce a great diversity of clinical disease. Some cause paralytic disease that may persist permanently (a typical feature of polioviruses), acute inflammation of the meninges with or without involvement of cerebral or spinal tissues, or sepsis-like illnesses in newborn infants. Inflammatory effects at other sites, such as the lungs, pleura, heart, and skin, have been also observed, often without concomitant or preceding central nervous system (CNS) involvement. Occasionally, infections may result in chronic, active disease processes.

## EPIDEMIOLOGY

Animals are not involved in human disease

Humans are the major natural host for the polioviruses, coxsackieviruses, and echoviruses. There are enteroviruses of other animals with limited host ranges that do not appear to extend to humans. Conversely, viruses thought to be identical or related to human enteroviruses have been isolated from dogs and cats. Whether these agents cause disease in such animals is debatable, and there is no evidence of spread from animals to humans.

The enteroviruses have a worldwide distribution, and asymptomatic infection is common. The proportion of infected individuals who develop illness varies from 2 to 100%, depending on the serotype or strain involved and the age of the patient. Secondary infections in households are common and range as high as 40 to 70%, depending on factors such as family size, crowding, and sanitary conditions.

Proportion of asymptomatic infections varies with strain

In some years, certain serotypes emerge as dominant epidemic strains; they then may wane, only to reappear in epidemic fashion years later. For example, echovirus 16 was a major cause of outbreaks in the eastern United States in 1951 and 1974. Coxsackievirus B1 was common in 1963; echovirus 9 in 1962, 1965, 1968, and 1969; and echovirus 30 in 1968 and 1969. The emergence of dominant serotypes is quite unpredictable from year to year. All enteroviruses show a seasonal predilection in temperate climates; epidemics are usually observed during the summer and fall months. In subtropical and tropical climates, the transmission may occur year-round.

Dominant epidemic stains come and go

Strong prevalence in summer and fall

Direct or indirect fecal–oral transmission is considered the most common mode of spread. After infection, the virus persists in the oropharynx for 1 to 4 weeks, and it can be shed in the feces for 1 to 18 weeks. Thus, sewage-contaminated water, fecally contaminated foods, or passive transmission by insect vectors (flies, cockroaches) may occasionally be the source of infection. More commonly, however, spread is directly from person to person. This mode of transmission is suggested by the high infection rates seen among young children, whose hygienic practices tend to be less than optimal, and in crowded households. Approximately two thirds of all isolates are from children 9 years of age or younger.

Person-to-person fecal–oral transmission correlates with predominance in children

Incubation periods vary, but relatively short intervals (2 to 10 days) are frequent. Often, illness is seen concurrently in more than one family member, and the clinical features vary within the household.

Incubation periods are typically short

## PATHOGENESIS

Initial binding of an enterovirus to the cell surface is commonly between an attachment protein in a "canyon" configuration on the virion surface and cell receptors belonging to the immunoglobulin gene superfamily. These receptors map to chromosome 19. A different receptor, belonging to the integrin group of adhesion molecules, has been identified for at least one echovirus serotype. Following attachment, the virion is enveloped by the cell membrane, and its RNA is released into the cellular cytoplasm where it binds to ribosomes and commences protein synthesis. Newly synthesized virions are released by lysis to spread to the other cells.

Initial attachment binds viral surface protein to cell surface receptors

Host receptor may relate to immunoglobulin or integrin families

After primary replication in epithelial cells and lymphoid tissues in the upper respiratory and gastrointestinal tracts, viremic spread to other sites can occur. Potential target organs vary according to the virus strain and its tropism, but may include the CNS, heart, vascular endothelium, liver, pancreas, lungs, gonads, skeletal muscles, synovial tissues, skin, and mucous membranes. Histopathologic findings include cell necrosis and mononuclear cell inflammatory infiltrates; in the CNS, the inflammatory cells are localized most prominently in perivascular sites. The initial tissue damage is thought to result from the lytic cycle of virus replication; secondary spread to other sites may ensue. Viremia is usually undetectable by the time symptoms appear, and termination of virus replication appears to correlate with the appearance of circulating neutralizing antibody, interferon, and mononuclear cell infiltration of infected tissue. The early dominant antibody response is with immunoglobulin M (IgM), which usually wanes 6 to 12 weeks after onset to be replaced progressively by increased IgG-specific antibodies. The important role of antibodies in termination of infection, demonstrated in mouse models of group B coxsackievirus infections, is supported by the observation of persistent echovirus and poliovirus replication in patients with antibody deficiency diseases.

Initial replication in epithelial and lymphoid cells is followed by viremic spread

Injury by cell lysis localized in perivascular sites

Antibody response terminates replication

Although initial acute tissue damage may be caused by the lytic effects of the virus on the cell, the secondary sequelae may be immunologically mediated. Enterovirus-caused poliomyelitis, disseminated disease of the newborn, aseptic meningitis, encephalitis, and acute respiratory illnesses, thought to represent primary lytic infections, can usually be identified through routine methods of virus isolation and determination of specific

In addition to lytic effects of virus there are probable immunopathologic manifestations

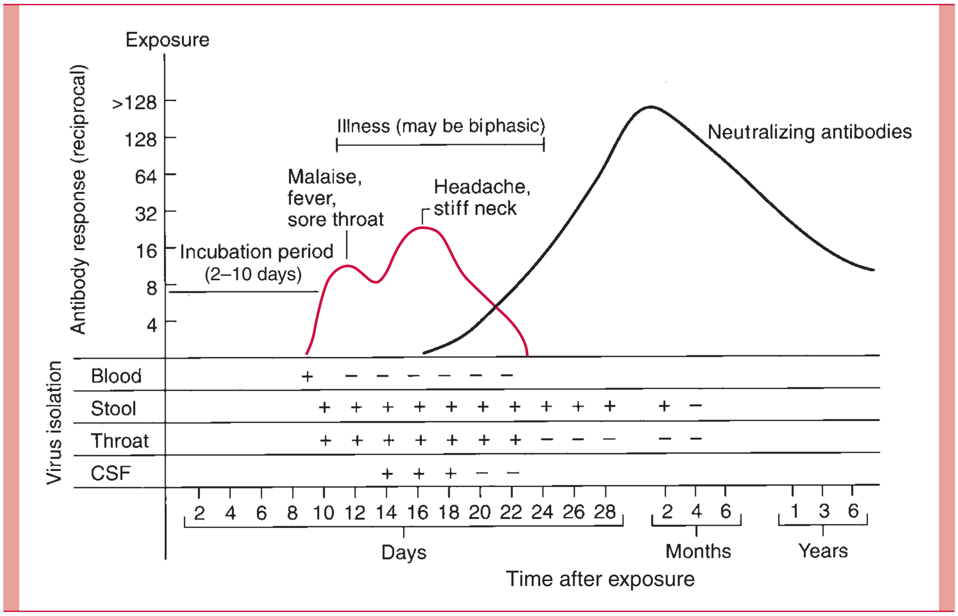


FIGURE 36–1
Antibody response and viral isolation from various sites in a typical case of enteroviral infection.

antibody titer changes. On the other hand, syndromes such as myopericarditis, nephritis, and myositis have been associated with enteroviruses primarily because of serologic and epidemiologic evidence. In many of these cases, viral isolation is the exception rather than the rule. The pathogenesis of these latter infections is not clear; however, observations suggest that the acute infectious phase of the virus may be mild or subclinical and often subsides by the time clinical illness becomes evident. Illness may represent a host immunologic response to tissue injury by the virus or to viral or virus-induced antigens that persist in the affected tissues. In experimental group B coxsackievirus myocarditis, mononuclear inflammatory cells (monocytes, natural killer lymphocytes) seem to play a greater role than antibody in termination of infection, and the persistence of inflammation after disappearance of detectable infectious virus or viral antigen appears to be mediated by cytotoxic T lymphocytes. Experimental findings have led to another hypothesis regarding pathogenic mechanisms, called **molecular mimicry.** This is best conceptualized as a form of virus-induced autoimmune response. It is known that small peptide sequences on viral epitopes can sometimes be shared by host tissues. Thus, an immune response produced by the virus may also generate antibodies or cytotoxic cross-reactive effector lymphocytes that recognize shared determinants located on host cells. For example, a monoclonal antibody directed against a neutralizing site of a group B coxsackievirus has also been shown to react strongly with normal myocardial cells.

Disease may follow the acute infection

Coxsackie B myocarditis may involve virus-induced cross reacting antibody

## IMMUNITY

Infection by a specific serotype in an immunologically normal host is followed by a humoral antibody response, which can often be detected by neutralization methods for many years thereafter (Fig 36–1). There is relative immunity to reinfection by the same serotype; however, reinfection has been reported, usually resulting in subclinical infection or mild illness.

Immunity is serotype specific

# ENTEROVIRUSES: CLINICAL ASPECTS

## DIAGNOSIS

In acute enterovirus-caused syndromes, diagnosis is most readily established by virus isolation from throat swabs, stool or rectal swabs, body fluids, and occasionally tissues.

Viremia is usually undetectable by the time symptoms appear. When there is CNS involvement, cerebrospinal fluid (CSF) cultures taken during the acute phase of the disease may be positive in 10 to 85% of cases (except in poliovirus infections, in which virus recovery from this site is rare), depending on the stage of illness and the viral serotype involved. Direct isolation of virus from affected tissues or body fluids in enclosed spaces (eg, pleural, joint, pericardial, or CSF) usually confirms the diagnosis. Isolation of an enterovirus from the throat is highly suggestive of an etiologic association; the virus is usually detectable at this site for only 2 days to 2 weeks after infection. Isolation of virus from fecal specimens only must be interpreted more cautiously; asymptomatic shedding from the bowel may persist for as long as 4 months (see Fig 36–1). The polymerase chain reaction with reverse transcription and complementary DNA amplification (RT-PCR) can also be used to detect enteroviral RNA sequences in tissues and body fluids, thus greatly enhancing diagnostic sensitivity and speed.

Viral isolation from pharynx or closed space is significant

Prolonged shedding in stool

RT-PCR enhances diagnostic sensitivity

The diagnosis may be further supported by fourfold or greater neutralizing antibody titer changes between paired acute and convalescent serum samples. However, this method is often expensive and cumbersome, requiring careful selection of serotypes for use in antigens. Quantitative interpretations of antibody titers on single serum samples are rarely helpful, because of the wide range of titers to different serotypes that can be found among healthy individuals.

Serodiagnosis is usually impractical

## TREATMENT AND PREVENTION

None of the currently available, approved antiviral agents has been shown effective in treatment or prophylaxis of enterovirus infections; however, the antipicornaviral drug, pleconaril (see Chapter 33) is currently being studied. Treatment is symptomatic and supportive. Vaccines for the prevention of poliovirus infections are discussed later in this chapter. Although proper disposal of feces and careful personal hygiene are recommended, the usual quarantine or isolation measures are relatively ineffective in controlling the spread of enteroviruses in the family or community.

Hygenic factors make prevention of spread difficult

# ENTEROVIRUSES: SPECIFIC GROUPS

## *Polioviruses*

POLIO

## EPIDEMIOLOGY

Worldwide, the most important enteroviruses are the three poliovirus serotypes (types 1, 2, and 3). They first emerged as important causes of disease in developed temperate zone countries during the latter part of the 19th century, and they have become increasingly important elsewhere as living conditions improve in developing countries. This somewhat paradoxical situation is related to the fact that the risk of paralytic disease resulting from infection increases with age. Improvement of sanitary conditions tends to impede spread of the viruses; thus, individuals may become infected not in early infancy but later in life, when paralysis is more likely to occur.

Risk of paralysis from infection increases with age

## PATHOGENESIS

The particular tropism of polioviruses for the CNS, which they usually reach by passage across the blood–CNS barrier, is perhaps favored by reflex dilatation of capillaries supplying the affected motor centers of the anterior horn of the brainstem or spinal cord. An

CNS tropism by blood or peripheral nerves

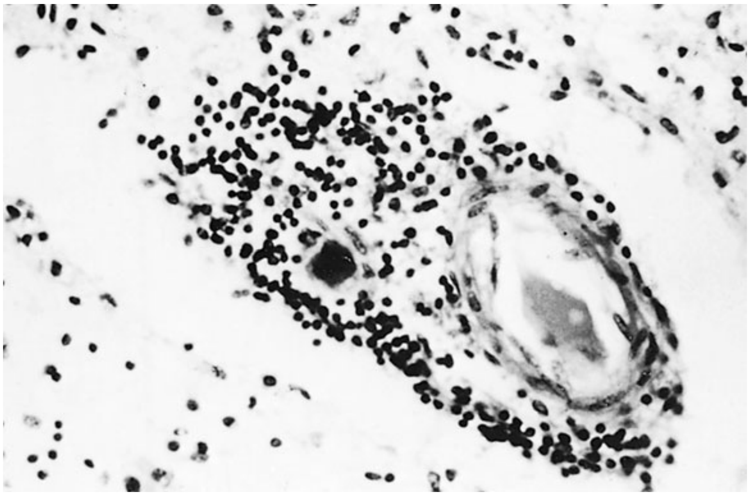

FIGURE 36–2
Section of spinal cord from a fatal case of poliomyelitis, demonstrating perivenous mononuclear cell inflammatory reaction. (*Courtesy of Dr. Peter C. Johnson.*)

Motor neuron cells destroyed

alternate pathway is via the axons or perineural sheaths of peripheral nerves. Motor neurons are particularly vulnerable to infection and variable degrees of neuronal destruction. The histopathologic findings in the brainstem and spinal cord include necrosis of neuronal cells and perivascular "cuffing" by infiltration with mononuclear cells, primarily lymphocytes (Fig 36–2).

# POLIO: CLINICAL ASPECTS

## MANIFESTATIONS

Subclinical and abortive poliomyelitis common

Aseptic meningitis recovers rapidly

Paralytic poliomyelitis manifests flaccid paralysis without sensory loss

Recovery of function up to 6 months

Most infections (perhaps 90%) are either completely subclinical or so mild that they do not come to attention. When disease does result, the incubation period ranges from 4 to 35 days, but is usually between 7 and 14 days. Three types of disease can be observed. Abortive poliomyelitis is a nonspecific febrile illness of 2- to 3-day duration with no signs of CNS localization. Aseptic meningitis (nonparalytic poliomyelitis) is characterized by signs of meningeal irritation (stiff neck, pain, and stiffness in the back) in addition to the signs of abortive poliomyelitis; recovery is rapid and complete, usually within a few days. Paralytic poliomyelitis, occurs in less than 2% of infections. It is the major possible outcome of infection and is often preceded by a period of minor illness, sometimes with two or three symptom-free days intervening. There are signs of meningeal irritation, but the hallmark of paralytic poliomyelitis is asymmetric flaccid paralysis, with no significant sensory loss. The extent of involvement varies greatly from case to case; however, in its most serious forms, all four limbs may be completely paralyzed or the brainstem may be attacked, with paralysis of the cranial nerves and muscles of respiration (bulbar polio). The maximum extent of involvement is evident within a few days of first paralysis. Thereafter, as temporarily damaged neurons regain their function, recovery begins and may continue for as long as 6 months; paralysis persisting after this time is permanent.

## PREVENTION

Two types of poliovirus vaccines are currently licensed in the United States: inactivated polio vaccine and live oral attenuated virus vaccine. Each contains all three viral serotypes.

Inactivated polio vaccine (IPV) was introduced in 1955; its use was associated with a dramatic decline in paralytic cases (Fig 36–3). Vaccination is by subcutaneous injection. Primary vaccination with three doses of the present enhanced-potency IPV (two doses 6–8 weeks apart and the third 8–12 months later) produces antibody responses in more

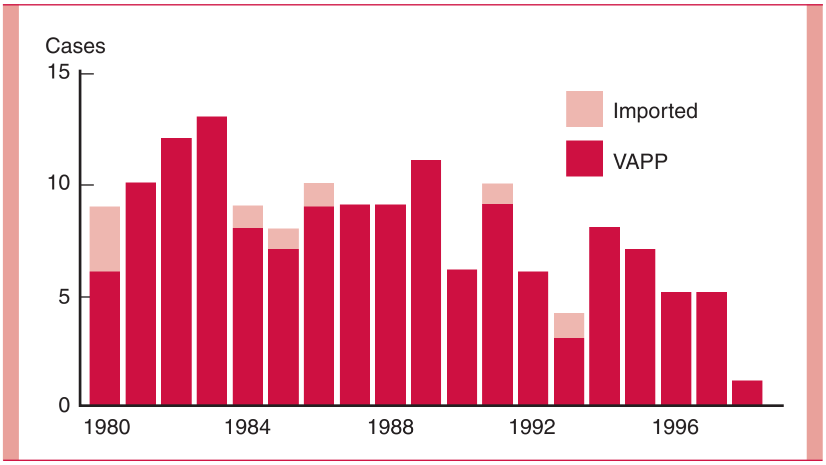


FIGURE 36–3
Total number of reported paralytic poliomyelitis cases and number of reported vaccine-associated cases (VAPP)—United States, 1950–1999. (*From the Centers for Disease Control and Prevention, 2000.*)

than 98% of recipients. The current product is considered quite safe, with no significant deleterious side effects. Inactivated (Salk) vaccine is used in many countries

Oral polio vaccine (OPV) is composed of live, attenuated viruses that have undergone serial passage in cell cultures from humans and subhuman primates. It was first licensed in the United States in 1963. The vaccine is given orally as a primary series of three doses (the first two doses usually 6–8 weeks apart and the third 8–12 months later) and produces antibodies to all three serotypes in more than 95% of recipients; these antibodies persist for several years. As with IPV, recall boosters are recommended to maintain adequate antibody levels. Like wild poliovirus, OPV viruses infect and replicate in the oropharynx and intestinal tract and can be spread to other persons.

Live (Sabin) vaccine is given orally (OPV)

Vaccine virus replicates and can spread

One disadvantage of OPV is the remote risk of vaccine-associated paralytic disease in some recipients or their household contacts, including immunocompromised persons. The incidence of vaccine-associated paralytic poliomyelitis is estimated at approximately 1 per 2.4 million doses distributed. Since the end of 1999, exclusive use of IPV has been recommended for all routine immunizations in the United States. OPV is recommended only in special circumstances (eg, an unvaccinated child who will be traveling in less than 4 weeks to an endemic area).

Vaccine-associated poliomyelitis is a remote risk with OPV

IPV is currently preferred

No cases of paralytic poliomyelitis attributed to indigenously acquired wild poliovirus have occurred in the United States since 1979, and the last case in the Western Hemisphere occurred in 1991. Nevertheless, it must be kept in mind that importation of these strains can readily occur from endemic areas in developing nations. Once introduced into a community, the virus can spread rapidly among susceptible individuals. Thus, continuing immunization programs are of utmost importance in preventing spread of this disease. In 1988, the World Health Organization resolved to eradicate polio from the world by the year 2000. Thus far, progress toward that goal has been hampered by political strife and severe poverty in many underdeveloped nations in Africa, Asia and the Middle East.

# *Coxsackieviruses and Echoviruses*

## EPIDEMIOLOGY

The coxsackieviruses and echoviruses are widespread throughout the world. Their epidemiology and pathogenesis are much the same as those of the polioviruses. Unlike polioviruses, they have a greater tendency to affect the meninges and occasionally the cerebrum, but only a few affect anterior horn cells.

Often do not affect motor neurons

The consequences of infection with these agents are highly variable and related only in part to virus subgroup and serotype. Up to 60% of infections are subclinical. The main

TABLE 36–2

**Clinical Syndromes and Commonly Associated Enterovirus Serotypes[a]**

| Syndrome | Coxsackievirus Group A | Coxsackievirus Group B | Echovirus and Enterovirus (E) |
|---|---|---|---|
| Aseptic meningitis, encephalitis | 2, 4, 7, **9,** 10 | 1, **2, 3, 4, 5** | **4, 6, 9, 11, 16, 30,** E70, E71 |
| Muscle weakness and paralysis (poliomyelitis-like disease) | 7, **9** | 2, 3, 4, 5 | 2, 4, 6, 9, 11, 18, 30, **E71** |
| Cerebellar ataxia | 2, 4, **9** | 3, 4 | 4, 6, 9 |
| Exanthems and enanthems | **4, 5, 6, 9, 10, 16** | 2, 3, 4, 5 | **2, 4, 5, 6, 9, 11, 16, 18, 25** |
| Pericarditis, myocarditis | 4, 16 | **2, 3, 4, 5** | 1, 6, 8, 9, 19 |
| Epidemic myalgia (pleurodynia), orchitis | 9 | 1, **2, 3, 4, 5** | 1, 6, 9, |
| Respiratory | 9, 16, **21,** 24 | 1, 3, 4, 5 | **4, 9, 11,** 20, 25 |
| Conjunctivitis | **24** | 1, 5 | 7, **E70** |
| Generalized disease (infants) | – | 1, **2, 3, 4, 5** | 3, 6, 9, 11, 14, 17, 19 |

[a] Serotypes most commonly associated with syndrome are in **boldface.**

Most infections subclinical

Wide range of clinical manifestations

interest in these agents stems from their ability to cause more serious illness, which becomes most evident during epidemics of infection with a particular agent. Inapparent infection is common. Illness manifestations vary from mild to lethal. Table 36–2 lists the major syndromes and serotypes commonly associated with each. However, considerable overlap occurs, and one should not be surprised if an enteroviral serotype found in connection with a specific syndrome differs from that most often encountered.

## MANIFESTATIONS

Aseptic meningitis most common syndrome

Aseptic meningitis is the most frequently recognized clinical illness associated with enterovirus infections. This syndrome can be mild and self-limiting, lasting 5 to 14 days; however, it is sometimes accompanied by encephalitis, which can lead to permanent neurologic sequelae.

Myocarditis often associated with group B coxsackieviruses

Acute inflammation of the heart muscle (myocarditis), its covering membranes (pericarditis), or both can be caused by a variety of viral agents. Group B coxsackieviruses are the most commonly implicated enteroviruses. Such infections are usually self-limiting but may be fatal in the acute phase (arrhythmia or heart failure) or progress to chronic dilated myocardiopathy.

Exanthems can mimic other diseases

Herpangina infection of palate and tonsils

The exanthems are often not associated with CNS inflammation. They can resemble rubella, roseola infantum, or adenoviral macular or maculopapular exanthems but may also appear as vesicular or hemangioma-like lesions. One interesting syndrome is hand-foot-and-mouth disease, which usually affects children and is characterized by a vesicular eruption over the extremities and the oral cavity. Coxsackie virus A16 is most commonly implicated, but others, such as enterovirus 71, can cause a similar illness. Herpangina is an enanthematous (mucous membrane–affecting) febrile disease in which small vesicles or white papules (lymphonodules) surrounded by a red halo are seen over the posterior palate, pharynx, and tonsillar areas. This mild, self-limiting (1 to 2 week) illness has usually been associated with infection by several different group A coxsackievirus serotypes.

Epidemic myalgia, with pleuritic pain

Epidemic myalgia (pleurodynia or Bornholm disease) is characterized by fever and sudden onset of intense upper abdominal or thoracic pain. The pain may be aggravated by movement, such as breathing or coughing, and can persist as long as 14 days. Group B coxsackieviruses are often implicated.

Generalized disease of the newborn is a disseminated, often lethal enteroviral infection characterized by pathologic changes in the heart, brain, liver, and other organs.

It is apparent from Table 36–2 that the spectrum of disease produced by these viruses is enormous and that many other illnesses may also result from infections by this subgroup. Epidemics of acute hemorrhagic keratoconjunctivitis associated with enterovirus 70 and localized outbreaks of disease resembling paralytic poliomyelitis caused by enterovirus 71 infection have been described. In addition, there is evidence that certain enteroviruses, particularly group B coxsackievirus serotypes, may sometimes participate in the pathogenesis of insulin-dependent diabetes mellitus, acute arthritis, polymyositis, and idiopathic acute nephritis. Further investigations are required to establish whether such associations are significant.

## ADDITIONAL READING

Cochi SL, Hull HF, Sutter RW, et al. Commentary: The unfolding story of global poliomyelitis eradication. *J Infect Dis* 1997;175:S1–3. This commentary and the papers that follow outline the remarkable strategies for global eradication and why they are expected to succeed.

Ho M, Chen E-R, Hsu K-H, et al. An epidemic of enterovirus 71 infection in Taiwan. *N Engl J Med* 1999;341:929–935. In 1998, a widespread epidemic of enterovirus 71 affected more than 100,000 persons in Taiwan. This report details the epidemiologic and often severe clinical features.

Rotbart HA. Enteroviral infectious of the central nervous system. *Clin Infect Dis* 1995;20:971–981. This paper describes the molecular pathogenesis, clinical diseases and diagnosis of enteroviral infections.

Starlin R, Reed N, Leeman B, et al. Acute flaccid paralysis syndrome associated with echovirus 19, managed with pleconaril and intravenous immunoglobulin. *Clin Infect Dis* 2001;33:730–732. This report describes the rationale for attempting newer treatments for severe enteroviral infections.

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CHAPTER 37

# Hepatitis Viruses

W. Lawrence Drew

The causes of hepatitis are varied and include viruses, bacteria, and protozoa, as well as drugs and toxins (eg, isoniazid, carbon tetrachloride, and ethanol). The clinical symptoms and course of acute viral hepatitis can be similar, regardless of etiology, and determination of a specific cause depends primarily on the use of laboratory tests. Hepatitis may be caused by at least five different viruses whose major characteristics are summarized in Table 37–1. *Non-A, non-B hepatitis* is a term previously used to identify cases of hepatitis not due to hepatitis A or B. With the discovery of the hepatitis viruses C, E, and G, virtually all the viral etiologies of non-A, non-B disease can be specifically identified. Other viruses, such as Epstein–Barr virus and cytomegalovirus, can also cause inflammation of the liver, but hepatitis is not the primary disease caused by them. Yellow fever is associated with hepatitis but is now uncommon.

## Hepatitis A

### VIROLOGY

Hepatitis A virus is an unenveloped, single-stranded RNA virus with cubic symmetry and a diameter of 27 nm (Fig 37–1). The virus resists inactivation and is stable at –20°C with low pH. These properties are similar to those of picornaviruses, and hepatitis A virus has now been classified in a separate genus of picornaviruses as a hepatovirus. There is only one serotype of hepatitis A virus. The virus has been successfully cultivated in primary marmoset liver cell cultures and in fetal rhesus monkey kidney cell cultures.

Only one serotype

### HEPATITIS A DISEASE

Clinical Capsule

Hepatitis A virus is the cause of what was formerly termed infectious hepatitis or short-incubation hepatitis. This virus is spread by the fecal–oral route, and outbreaks may be associated with contaminated food or water. The illness is subclinical in up to one half of infected adults. When symptomatic, there is usually fever and jaundice. Although fatal disease may occur, self-limited illness is the rule. Chronic hepatitis A rarely if ever occurs.

TABLE 37–1

**Comparison of A, B, D (Delta), C, and E Hepatitis**

| Feature | A | B | D | C[a] | E |
|---|---|---|---|---|---|
| Virus type | Single-stranded RNA | Double-stranded DNA | Single-stranded RNA | RNA | RNA |
| Percent of viral hepatitis | 50 | 41 | <1 | 5 | <1 |
| Incubation period (days) | 15–45 (mean, 25) | 7–160 (mean, 60–90) | 28–45 | 15–160 (mean, 50) | ? |
| Onset | Usually sudden | Usually slow | Variable | Insidious | ? |
| Age preference | Children, young adults | All ages | All ages | All ages | Young adult |
| Transmission | | | | | |
| Fecal–oral | +++ | ± | ± | − | +++ |
| Sexual | + | ++ | ++ | + | +? |
| Transfusion | − | ++ | +++ | +++ | − |
| Severity | Usually mild | Moderate | Often severe | Mild | Variable |
| Chronicity (%) | None | 10 | 50–70 | >50% | None |
| Carrier state | None | Yes | Yes | Yes | ? |
| Immune serum globulin protective | Yes | Yes[b] | Yes[c] | Uncertain | ? |

*Abbreviation*: Plus and minus signs indicate relative frequencies.

[a] Many individuals with hepatitis C virus are also infected with the hepatitis G virus, which is similar to hepatitis C.

[b] Hyperimmune globulin more protective.

[c] Prevention of hepatitis B prevents hepatitis D.

## EPIDEMIOLOGY

No chronic carriage

Humans appear to be the major natural hosts of hepatitis A virus. Several other primates (including chimpanzees and marmosets) are susceptible to experimental infection, and natural infections of these animals may occur. The major mode of spread of hepatitis A is fecal–oral. Inoculation of infectious material intramuscularly can produce disease; transmission through blood transfusion, although possible, is not an important means of spread. While most cases of hepatitis A are not linked to a single contaminated source and occur sporadically, outbreaks have been described. The disease is common under conditions of crowding, and it occurs at high frequency in mental hospitals, schools for the retarded, and day-care centers. A chronic carrier state has not been observed with hepatitis A; perpetuation of the virus in nature presumably depends on sporadic subclinical infections and

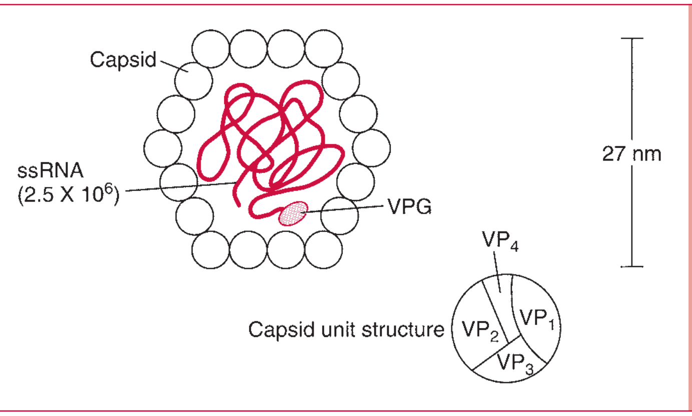


FIGURE 37–1
Diagram of the proposed structure of the hepatitis A virus. The protein capsid is made up of four viral polypeptides ($VP_1$ to $VP_4$). Inside the capsid is a single-stranded (ss) molecule of RNA (molecular weight 2.5 + 106), which has a genomic viral protein (VPG) on the 5′ end. (*Reprinted with permission of Dr. J. H. Hoofnagle and of Abbot Laboratories, Diagnostic Division, North Chicago, Illinois.*)

person-to-person transmission. Outbreaks of hepatitis A have been linked to the ingestion of undercooked seafood, usually shellfish from waters contaminated with human feces. Common-source outbreaks related to other foods, including vegetables as well as contaminated drinking water, have also been reported.

Hepatitis A is widespread but seroepidemiologic studies have shown marked variation in infection rates among various population groups. For example, rates are higher among those of lower socioeconomic status and among male homosexuals. Less than 50% of the general population of the United States now has serologic evidence of prior hepatitis A virus infection, and rates have been decreasing since 1970, apparently because of better sanitation and less crowding. In contrast, more than 90% of the adult population in many developing countries shows evidence of previous hepatitis A infection. The risk of clinically evident disease is much higher in infected adults than in children; travelers from developed countries who enter endemic areas are particularly susceptible. Patients are most contagious in the 1 to 2 weeks prior to the onset of clinical disease.

Subclinical infection is common in children

## PATHOGENESIS

The virus is believed to replicate initially in the enteric mucosa. It can be demonstrated in feces by electron microscopy for 10 to 14 days before onset of disease. In most patients with symptoms of the disease, virus is no longer found in fecal specimens. Multiplication in the intestines is followed by a period of viremia with spread to the liver. The response to replication in the liver consists of lymphoid cell infiltration, necrosis of liver parenchymal cells, and proliferation of Kupffer cells. The extent of necrosis often coincides with the severity of disease. A variable degree of biliary stasis may be present. Detectable levels of IgG antibody to hepatitis A virus persist indefinitely in serum, and patients with anti–hepatitis A virus antibodies are immune to reinfection. Although virus-specific IgA has been demonstrated in stool, secretory immunity has not been shown to be important for hepatitis A.

Contagion is greatest 10–14 days before symptoms appear

IgG-specific antibody is protective

# HEPATITIS A DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

In hepatitis A virus infection, an incubation period of 10 to 50 days (mean, 25 days) is usually followed by the onset of fever; anorexia (poor appetite); nausea; pain in the right upper abdominal quadrant; and, within several days, jaundice. Dark urine and clay-colored stools may be noticed by the patient 1 to 5 days before the onset of clinical jaundice. The liver is enlarged and tender, and serum aminotransferase and bilirubin levels are elevated as a result of hepatic inflammation and damage. Recovery occurs in days to weeks.

Fever, anorexia, and jaundice are common

Many persons who have serologic evidence of acute hepatitis A infection are asymptomatic or only mildly ill, without jaundice (anicteric hepatitis A). The infection-to-disease ratio is dependent on age; it may be as high as 20:1 in children and approximately 4:1 in older adults. Almost all cases (99%) of hepatitis A are self-limiting. Chronic hepatitis such as that seen with hepatitis B is very rare. In rare cases, fulminant fatal hepatitis associated with extensive liver necrosis may occur (~0.1%).

Chronic infection is rare

## DIAGNOSIS

Antibody to hepatitis A virus can be detected during early illness, and most patients with symptoms or signs of acute hepatitis A already have detectable antibody in serum. Early antibody responses are predominantly IgM, which can be detected for several weeks or months. During convalescence, antibody of the IgG class predominates. The best method for documentation of acute hepatitis A virus infection is the demonstration of high titers of virus-specific IgM antibody in serum drawn during the acute phase of illness. Because IgG antibody persists indefinitely, its demonstration in a single serum sample is not

IgM-specific antibody denotes acute infection indicative of recent infection; a rise in titer between acute and convalescent sera must be documented. Immune electron microscopic identification of the virus in fecal specimens and isolation of the virus in cell cultures remain research tools.

## TREATMENT AND PREVENTION

There is no specific treatment for patients with acute hepatitis A. Supportive measures include adequate nutrition and rest. Avoidance of exposure to contaminated food or water are important measures to reduce the risk of hepatitis A infection.

### Passive Immunization

Immune serum globulin provides temporary protection

Passive (ie, antibody) prophylaxis for hepatitis A has been available for many years. Immune serum globulin (ISG), manufactured from pools of plasma from large segments of the general population, is protective if given before or during the incubation period of the disease. It has been shown to be about 80 to 90% effective in preventing clinically apparent type A hepatitis. In some cases, infection occurs but disease is ameliorated; that is, patients develop anicteric, usually asymptomatic, hepatitis A. At present, ISG should be administered to household contacts of hepatitis A patients and those known to have eaten uncooked foods prepared or handled by an infected individual. Once clinical symptoms have appeared, the host is already producing antibody, and administration of ISG is not indicated. Persons from areas of low endemicity traveling to areas with high infection rates may receive ISG before departure and at 3- to 4-month intervals as long as potential heavy exposure continues, but active immunization is preferable (see below).

### Active Immunization

Inactivated virus vaccine confers long-term protection

For hepatitis A, live attenuated vaccines have been evaluated but have demonstrated poor immunogenicity and have not been effective when given orally. Formalin-killed vaccines induce antibody titers similar to those of wild-virus infection and are almost 100% protective. Use of this vaccine is preferable to passive prophylaxis for those with prolonged or repeated exposure to hepatitis A.

# *Hepatitis B*

## VIROLOGY

## STRUCTURE

Enveloped DNA virus

Hepatitis B virus is an enveloped DNA virus belonging to the family Hepadnaviridae. It is unrelated to any other human virus; however, related hepatotropic agents have been identified in woodchucks, ground squirrels, and kangaroos. A schematic of the hepatitis B virus is illustrated in Figure 37–2. The complete virion is a 42-nm, spherical particle that consists of an envelope around a 27-nm core. The core comprises a nucleocapsid that contains the DNA genome.

Smallest known human DNA virus

The viral genome consists of partially double-stranded DNA with a short, single-stranded piece. It comprises 3200 nucleotides, making it the smallest DNA virus known. Closely associated with the viral DNA is a DNA polymerase. Other components of the core are a hepatitis B core antigen (HBcAg) and the hepatitis B e antigen (HBeAg), which is a low-molecular-weight glycoprotein.

HBsAg is produced in great abundance

The envelope of the virus contains the hepatitis B surface antigen (HBsAg), which is composed of one major and two other proteins. Antigenically there exist a group-specific determinant, termed *a,* and a number of subtypes that are important in epidemiologic typing, but not in immunity, because there is antigenic cross-reactivity and cross-protection

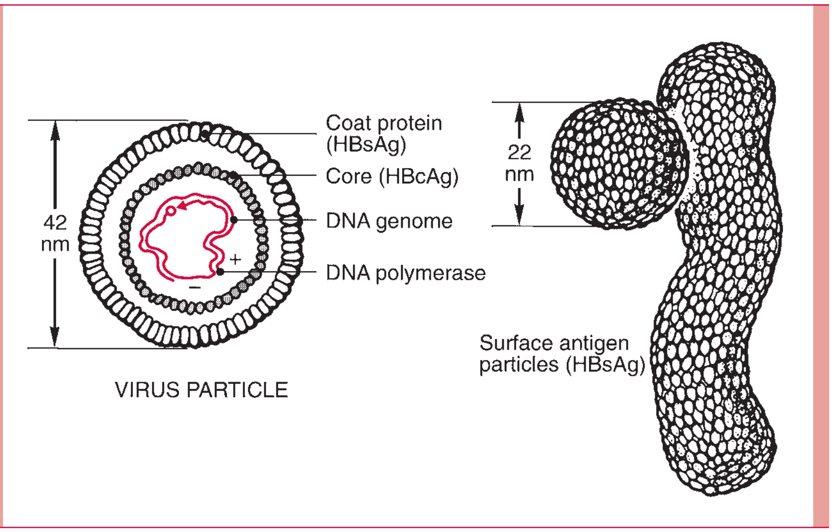


FIGURE 37–2
Schematic diagram of hepatitis B virion. The 42-nm particle is the "Dane particle" or the hepatitis B virus. The 22-nm particles are the filamentous and circular forms of hepatitis B surface antigen (HbsAg) or protein coat.

between subtypes. Aggregates of HBsAg are often found in great abundance in serum during infection. They may assume spherical or filamentous shapes with a mean diameter of 22 nm and may contain portions of the nucleocapsid (see Fig 37–2). Hepatitis B DNA can also be detected in serum and is an indication that infectious virions are present there. In infected liver tissue, evidence of HBcAg, HBeAg, and hepatitis B DNA is found in the nuclei of infected hepatocytes, whereas HBsAg is found in cytoplasm.

Found in cytoplasm of infected hepatocytes

## REPLICATION CYCLE

The replication of hepatitis B virus involves a reverse transcription step, and, as such, is unique among DNA viruses. The double-stranded DNA is organized as two strands. One, a short strand, is associated with the viral DNA polymerase and is of positive polarity. The complete or long strand is complementary and thus of negative polarity. In viral replication, full-length positive viral RNA transcripts are inserted into maturing core particles late in the replicative cycle. These mRNA strands form a template for a reverse transcription step in which negatively stranded DNA is synthesized. The RNA template strands are then degraded by ribonuclease activity. A positive-stranded DNA is then synthesized, although this is not completed prior to virus maturation and release and thus results in the variable-length short positive DNA strands found in the virions.

Unique replication using a reverse transcriptase step

Despite extensive attempts, hepatitis B virus has not been propagated in the laboratory. Humans appear to be the major host; however, as with hepatitis A, infection of subhuman primates has been accomplished experimentally.

Humans are the major hosts

# HEPATITIS B DISEASE

CLINICAL CAPSULE

Hepatitis B virus is the cause of what was formerly known as "serum hepatitis." This name was used to distinguish it from "infectious hepatitis" and reflected the association of this form of hepatitis with needle use or blood transfusion. Hepatitis B is usually an asymptomatic or limited illness with fever and jaundice for days to weeks. It becomes chronic in up to 10% of patients and may lead to cirrhosis or hepatocellular carcinoma.

## EPIDEMIOLOGY

Hepatitis B infection is found worldwide, with prevalence rates varying markedly between countries. Chronic carriers constitute the main reservoir of infection: in some

Chronic carriers are common in the Far East

countries particularly in the far East, as many as 5 to 15% of all persons carry the virus and most are asymptomatic. About 10% of patients with human immunodeficiency virus (HIV) infection are chronic carriers of hepatitis B.

Needlestick transmission is a risk for health care workers

In the United States, it is estimated that 1.5 million people are infected with hepatitis B and that 300,000 new cases occur annually. About 300 of these patients die of acute fulminant hepatitis, and 5–10% of infected patients become chronic hepatitis B virus carriers. As many as 4000 people die yearly of hepatitis B–related cirrhosis, and 1000 die of hepatocellular carcinoma. Approximately 50% of infections in the United States are sexually transmitted, and the prevalence of HBsAg in serum is higher in certain populations, such as male homosexuals, patients on hemodialysis or immunosuppressive therapy, patients with Down's syndrome, and injection drug users. Routine screening of blood donors for HBsAg has markedly decreased the incidence of posttransfusion hepatitis B. Multiple-pool blood products still cause occasional cases. Exposure to hepatitis viruses from direct contact with blood or other body fluids, probably through needlestick injuries, has resulted in a higher risk of hepatitis B in medical personnel. Attack rates are also high in spouses and sexual partners of infected patients.

Vertical transmission usually occurs during birth process

Hepatitis B infection of infants does not appear to be transplacentally transmitted to the fetus in utero but is acquired during the birth process by the swallowing of infected blood or fluids or through abrasions. The rate of virus acquisition is high (up to 90%) in infants born to mothers with acute hepatitis B infection or carrying HBsAg and HBeAg. Most infants do not develop clinical disease; however, infection in the neonatal period is associated with failure to produce antibody to HbsAg, allowing chronic carriage to occur in nearly 100% with perpetuation of transmission in the family setting.

Strong association between chronic infection and hepatocellular carcinoma

Hepatocellular carcinoma has been strongly associated with persistent carriage of hepatitis B virus by serologic tests and by detection of viral nucleic acid sequences integrated in tumor cell genomes. In many parts of Africa and Asia, primary liver cancer accounts for 20 to 30% of all types of malignancies, but in North and South America and Europe, only 1 to 2%. The estimated risk of developing the malignancy for persons with chronic hepatitis B is increased between 10- to more than 300-fold in different populations.

## PATHOGENESIS

Virus found in blood, saliva, and semen

In the past, hepatitis B was known as posttransfusion hepatitis or as hepatitis associated with the use of illicit parenteral drugs (serum hepatitis). However, over the past few years it has become clear that the major mode of acquisition is through close personal contact with body fluids of infected individuals. HBsAg has been found in most body fluids, including saliva, semen, and cervical secretions. Under experimental conditions, as little as 0.0001 mL of infectious blood has produced infection. Transmission is therefore possible by vehicles such as inadequately sterilized hypodermic needles or instruments used in tattooing and ear piercing.

Antibody to HBsAg is protective

The factors determining the different clinical manifestations of acute hepatitis B are largely unknown; however, some appear to involve immunologic responses of the host. The serum sickness–like rash and arthritis that may precede the development of symptoms and jaundice appear to be related to circulating immune complexes that activate the complement system. Antibody to HBsAg is protective and associated with resolution of the disease. Cellular immunity also may be important in the host response, because patients with depressed T-lymphocyte function have a high frequency of chronic infection with the hepatitis B virus. Antibody to the HBcAg, which appears during infection, is present in chronic carriers with persistent hepatitis B virion production and does not appear to be protective.

Chronic infection leads to progressive fibrosis and cirrhosis

The morphologic lesions of acute hepatitis B resemble those of other hepatitis viruses. In chronic active hepatitis B, the continued presence of inflammatory foci of infection results in necrosis of hepatocytes, collapse of the reticular framework of the liver, and progressive fibrosis. The increasing fibrosis can result in the syndrome of postnecrotic hepatic cirrhosis.

Integrated hepatitis B viral DNA can be found in nearly all hepatocellular carcinomas. The virus has not been shown to possess a transforming gene but may well activate a cellular oncogene. It is also possible that the virus does not play such a direct molecular role in oncogenicity, because the natural history of chronic hepatitis B infection involves cycles of damage or death of liver cells interspersed with periods of intense regenerative hyperplasia. This significantly increases the opportunity for spontaneous mutational changes that may activate cellular oncogenes. Whatever the mechanism, the association between chronic viral infection and hepatocellular carcinoma is clear, and liver cancer is a major cause of disease and death in countries in which chronic hepatitis B infection is common. The proven success of combined active and passive immunization in aborting hepatitis B infection in infancy or childhood makes hepatocellular carcinoma of the liver a potentially preventable disease.

Mechanism of hepatocellular carcinoma development is not clearly known

# HEPATITIS B DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

The clinical picture of hepatitis B is highly variable. The incubation period may be as brief as 7 days or as long as 160 days (mean, approximately 10 weeks). Acute hepatitis B is usually manifested by the gradual onset of fatigue, loss of appetite, nausea and pain, and fullness in the right upper abdominal quadrant. Early in the course of disease, pain and swelling of the joints and occasional frank arthritis may occur. Some patients develop a rash. With increasing involvement of the liver, there is increasing cholestasis and, hence, clay-colored stools, darkening of the urine, and jaundice. Symptoms may persist for several months before finally resolving.

Average incubation period is 10 weeks; range 7–160 days

In general, the symptoms associated with acute hepatitis B are more severe and more prolonged than those of hepatitis A; however, anicteric disease and asymptomatic infection occur. The infection-to-disease ratio, which varies according to age and method of acquisition, has been estimated to be approximately 6:1 or 7:1. Fulminant hepatitis, leading to extensive liver necrosis and death, develops in less than 1% of cases. One important difference between hepatitis A and hepatitis B is the development of chronic hepatitis. This occurs in approximately 10% of all patients with hepatitis B infection, but the risk is much higher for newborns (~100%), children (50%) and the immunocompromised. Chronic infection is associated with ongoing replication of virus in the liver and usually with the presence of HBsAg in serum. Chronic hepatitis may lead to cirrhosis, liver failure, or hepatocellular carcinoma, in up to 25% of patients.

Chronic hepatitis is most common with infection in early infancy or childhood

## DIAGNOSIS

The nomenclature of hepatitis B antigens and antibodies is shown in Table 37–2 and the sequence of their appearance in Figure 37–3. During the acute episode of disease, when there is active viral replication, large amounts of HBsAg and hepatitis B virus DNA can be detected in the serum, as can fully developed virions and high levels of DNA polymerase and HBeAg. Although HBcAg is also present, antibody against it invariably occurs and prevents its detection. With resolution of acute hepatitis B, HBsAg and HBeAg disappear from serum with the development of antibodies (anti-HBs and anti-HBe) against them. The development of anti-HBs is associated with elimination of infection and protection against reinfection. Anti-HBc is detected early in the course of disease and persists in serum for years. It is an excellent epidemiologic marker of infection, but is not protective. The laboratory diagnosis of acute hepatitis B is best made by demonstrating the IgM antibody to hepatitis B core antigen in serum. Almost all patients who develop jaundice are anti-HBc IgM positive at the time of clinical presentation. HBsAg may also be detected in serum. Past infection with hepatitis B is best determined by detecting IgG anti-HBc, anti-HBs, or both.

Appearance of anti-HBs signals elimination of infection

Acute infection associated with appearance of anti-HBc IgM

TABLE 37–2

**Nomenclature for Hepatitis B Virus Antigens and Antibodies**

| Abbreviation | Description |
|---|---|
| HBV | Hepatitis B virus; 42-nm double-stranded DNA virus; Dane particle |
| HBsAg | Hepatitis B surface antigen; found on surface of virus; formed in excess and seen in serum as 22-nm spherical and tubular particles; four subdeterminants (*adw, ayw, adr,* and *ayr*) identified |
| HBcAg | Core antigen (nucleocapsid core); found in nucleus of infected hepatocytes by immunofluorescence |
| HBeAg | Glycoprotein; associated with the core antigen; used epidemiologically as marker of potential infectivity; seen only when HBsAg is also present |
| Anti-HBs | Antibody to HBsAg; correlated with protection against and/or resolution of disease; used as marker of past infection or vaccination |
| Anti-HBc | Antibody to HBcAg; seen in acute infection and chronic carriers; anti-HBc IgM used as indicator of acute infection; anti-HBc IgG used as marker of past or chronic infection; apparently not important in disease resolution; does not develop in response to vaccine |
| Anti-HBe | Antibody to HBeAg |

Chronic infection associated with HBsAg persistence and no development of anti-HBs

In patients with chronic hepatitis B, evidence of viral persistence can be found in serum (Figure 37–4). HBsAg can be detected throughout the active disease process and anti-HBs does not develop, which probably accounts for the chronicity of the disease. However, anti-HBc is detected. Two types of chronic hepatitis can be distinguished. In one, HBsAg is detected but not HBeAg; these patients usually show minimal evidence of liver dysfunction. In the other, both antigens are found; the process is more active, with continued hepatic damage that may result in cirrhosis. Chronic infection with hepatitis B is best detected by persistence of HBsAg in blood for more than 6 to 12 months.

## TREATMENT

There is no specific treatment for acute hepatitis B. A high-calorie diet is desirable. Corticosteroid therapy has no value in uncomplicated acute viral hepatitis, and recent

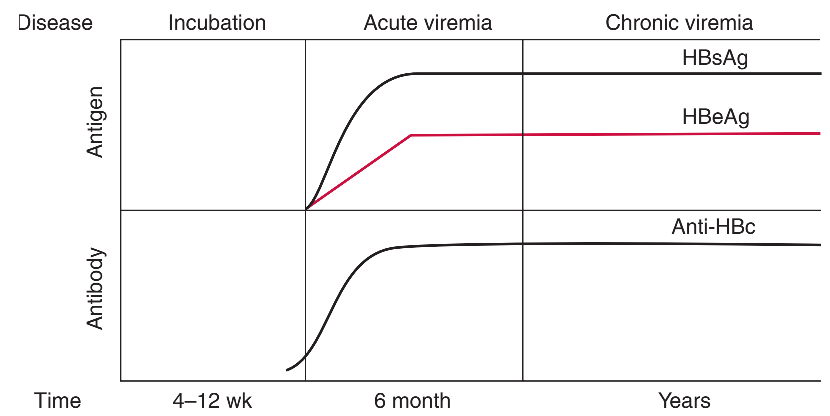


FIGURE 37–3
Sequence of appearance of viral antigens and antibodies in acute self-limiting cases of hepatitis B. HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; anti-HBc, antibody to hepatitis B core antigen; anti-HBe, antibody to HBeAg; anti-HBs, antibody to HBsAg.

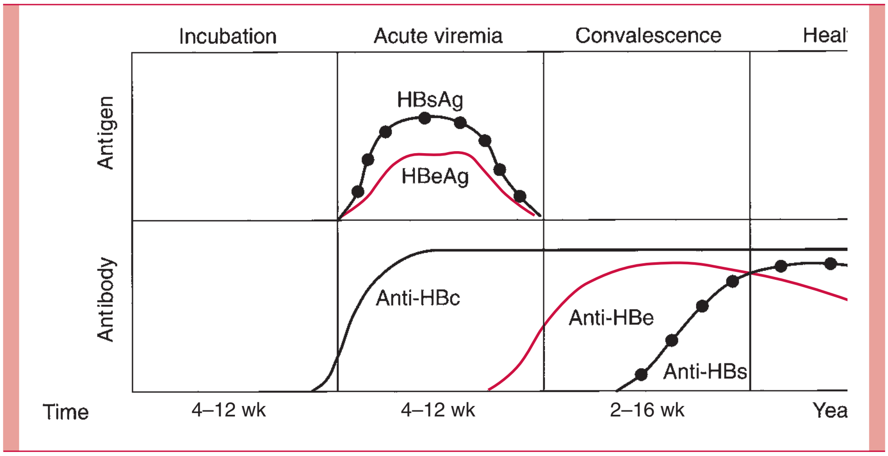


FIGURE 37–4
Sequence of appearance of viral antigens and antibodies in chronic active hepatitis B. HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; anti-HBc, antibody to hepatitis B core antigen; Antibodies to HBsAg and HBeAg not detected.

studies suggest that it may increase the severity of chronic hepatitis caused by hepatitis B virus. For chronic hepatitis B diseases, interferon alpha 10 million units three times weekly for 4 months provides long-term benefit in a minority (~33%) of patients, usually those who already demonstrate an acute immune response with low serum viral DNA levels. Lamivudine (3TC), a potent inhibitor of HIV is also active versus hepatitis B virus both in vitro and in initial clinical trials, but resistance to this agent develops in about 25% of patients after 12 months of therapy. Adefovir, a nucleotide analog of adenosine monophosphate, is newly approved for the treatment of chronic hepatitis B. Treatment should be considered for patients exhibiting chronic hepatitis B for more than 6 months with detectable serum levels of HBsAg, HBcAg, and hepatitis B DNA.

No specific treatment for acute infection

Interferon alpha lamivudine and adefovir are of benefit.

## PREVENTION

Safe sex practices and avoidance of needlestick injuries or injection drug use are approaches to diminishing the risk of hepatitis B infection. Both active prophylaxis and passive prophylaxis of hepatitis B infection can be accomplished. Most preparations of ISG contain only moderate levels of anti-HBs; however, specific hepatitis B immune globulin (HBIG) with high titers of hepatitis B antibody is now available. HBIG is prepared from sera of subjects who have high titers of antibody to HBsAg but are free of the antigen itself. Administration of HBIG soon after exposure to the virus greatly reduces the development of symptomatic disease. Postexposure prophylaxis with HBIG should be followed by active immunization with vaccine. Inactivated hepatitis B vaccines have been available for several years. The first was developed by purification and inactivation of HBsAg from the blood of chronic carriers, but this is no longer in use. The current vaccine is a recombinant product derived from HBAg grown in yeast. Excellent protection has been shown in studies on homosexual men and medical personnel. These groups and others, such as laboratory workers and injection drug users who come into contact with blood or other potentially infected materials, should receive hepatitis B vaccine as the preferred method of preexposure prophylaxis. Recently, immunization of all children has been recommended.

Postexposure treatment with HBIG temporarily reduces risk

Recombinant vaccine recommended for children and high-risk persons

A combination of active and passive immunization is the most effective approach to prevent neonatal acquisition and the development of chronic carriage in the neonate. Most hospitals recommend routine screening of pregnant women for the presence of HBsAg. Infants born to those who are positive should receive HBIG in the delivery room followed by three doses of hepatitis B vaccine beginning 24 hours after birth. A similar combination of passive and active immunization is used for unimmunized persons who have been exposed by needlestick or similar injuries. The procedure varies depending on the hepatitis B status of the “donor” case linked to the injury.

Combination of HBIG and vaccine significantly reduces vertical transmission

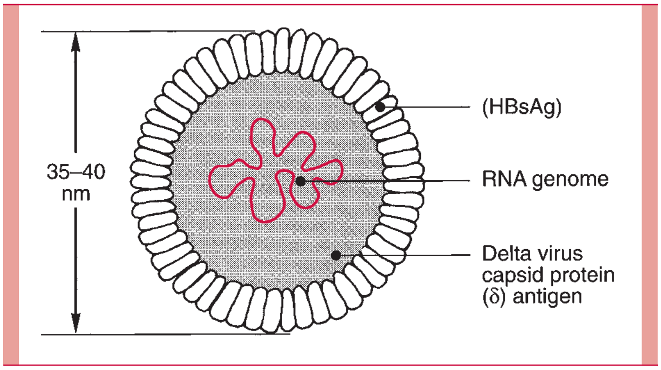


FIGURE 37–5
Schematic of delta hepatitis virus. Note outer layer derived from hepatitis B surface antigen (HBsAg).

## DELTA HEPATITIS (HEPATITIS D)

### VIROLOGY

Hepatitis D is found only in hepatitis B–infected persons

Virus uses HBsAg for assembly

Delta hepatitis is caused by the hepatitis D virus. This small single-stranded RNA virus requires the presence of hepatitis B surface antigens for its transmission and is thus found only in persons with acute or chronic hepatitis B infection. Strategies directed at preventing hepatitis B are also effective in preventing delta hepatitis. The method of replication of hepatitis D viral RNA is not clear. Associated with the RNA are proteins of 27 and 29 kilodaltons that constitute the delta antigen. This protein–RNA complex is surrounded by HBsAg (Fig 37–5). Thus, although the delta virus produces its own antigens, it co-opts the HBsAg in assembling its coat.

### DELTA HEPATITIS DISEASE

Greatest risk is among injection drug abusers

Delta hepatitis is most prevalent in groups at high risk of hepatitis B. Injection drug users are those at greatest risk in the western parts of the world, and as many as 50% of such individuals may have IgG antibody to the delta virus antigen. Other risks include dialysis. Nonparenteral and vertical transmission can also occur.

### DELTA HEPATITIS DISEASE: CLINICAL ASPECTS

#### MANIFESTATIONS

Simultaneous hepatitis B and D infections cause more severe disease

Two major types of delta infection have been noted: simultaneous delta and hepatitis B infection or delta superinfection in those with chronic hepatitis B. Simultaneous infection with both delta and hepatitis B results in clinical hepatitis that is indistinguishable from acute hepatitis A or B; however, fulminant hepatitis is much more common than with hepatitis B virus alone. Persons with chronic hepatitis B who acquire infection with hepatitis D suffer relapses of jaundice and have a high likelihood of developing chronic cirrhosis. Epidemics of delta infection have occurred in populations with a high incidence of chronic hepatitis B and have resulted in rapidly progressive liver disease, causing death in up to 20% of infected persons.

## DIAGNOSIS

Diagnosis is made most commonly by demonstrating IgM or IgG antibodies, or both, to the delta antigen in serum. IgM antibodies appear within 3 weeks of infection and persist for several weeks. IgG antibodies persist for years.

Diagnosis is by detection of antibodies

## TREATMENT AND PREVENTION

Response to treatment with interferon alpha in patients with delta hepatitis (and hepatitis B) is less than in those with hepatitis B alone. Recommended doses are higher and may produce sustained improvement in only 15–25% of patients.

Because the capsid of delta hepatitis is HBsAg, measures aimed at limiting the transmission of hepatitis B (eg, vaccination, blood screening) prevent the transmission of delta hepatitis. Individuals infected with hepatitis B or D should not donate blood, organ, tissues, or semen. Safe sex should be practiced unless there is only a single sex partner who is already infected. Methods of reducing transmission include decreased use of contaminated needles and syringes by injection drug users and use of needle safety devices by health care workers.

Major for prevention of hepatitis B also prevent hepatitis

# HEPATITIS C

It is now known that a large number of hepatitis cases are due to an RNA virus termed hepatitis C virus. Its existence and role in the etiology of hepatitis was identified by preparing numerous complementary DNA clones from the presumed RNA virus in infectious serum. Peptides encoded by these clones were then tested for reaction with sera from cases of hepatitis and one was found to be highly specific, providing a basis for a serologic test.

Cause identified by molecular cloning techniques

## VIROLOGY

Hepatitis C virus is an RNA virus in the flavivirus (eg, yellow fever, dengue) family. It has a very simple genome, consisting of just three structural and five nonstructural genes. There are at least six major genotypes, with multiple subtypes. The genotypes have different geographic distributions and may be associated with differing severity of disease as well as response to therapy.

RNA virus, with six major genotypes

## HEPATITIS C DISEASE

CLINICAL CAPSULE

Hepatitis C is an insidious disease in that it does not usually cause a clinically evident acute illness. Instead, its first manifestation (in 25% of those infected) may be the presence of smoldering chronic hepatitis that may ultimately lead to liver failure. Its transmission is less well understood than for hepatitis A, B, and D. Hepatitis C was the major cause of posttransfusion hepatitis until a serologic test for screening blood donors was developed.

The transmission of hepatitis C by blood is well documented: indeed, until screening blood for transfusions was introduced, it caused the great majority of cases of posttransfusion hepatitis. Hepatitis C may be sexually transmitted but to a much lesser degree than hepatitis B. Needle sharing accounts for up to 40% of cases. In the United States, 3.5 million

Major transmission was from blood and blood products but is now from “needle sharing” people (1.8%) have antibody to hepatitis C. Screening of donor blood for antibody has reduced posttransfusion hepatitis by 80–90%. Since the 1980s, outbreaks of hepatitis C have been associated with IVIG. To reduce this risk, all US-licensed IGIV products now have additional viral inactivation steps included in the manufacturing process. Furthermore, all immunoglobulin products (including intramuscular immunoglobulin products that have not been associated with hepatitis C) that lack viral inactivation steps are now excluded if hepatitis C virus is detected by polymerase chain reaction (PCR). Other individuals considered at risk for hepatitis C are chronic hemodialysis patients and spouses.

## HEPATITIS C DISEASE: CLINICAL ASPECTS

### MANIFESTATIONS

Acute illness usually not apparent

Chronic infection is common

The incubation period of hepatitis C averages 6–12 weeks. The infection is usually asymptomatic or mild and anicteric but results in a chronic carrier state in up to 85% of adults of patients. The average time from infection to the development of chronic hepatitis is 10–18 years. Cirrhosis and hepatocellular carcinoma are late sequelae of chronic hepatitis. Chronic hepatitis tends to wax and wane, is often asymptomatic, and may be associated with either elevated or normal alanine aminotransferase (ALT) values in serum. Chronic hepatitis C is the leading cause of liver transplantation in the United States.

### DIAGNOSIS

Antibody responses are usually delayed

Hepatitis C RNA can be detected and quantitated by PCR

Antigens of hepatitis C are not detectable in blood, so diagnostic tests attempt to demonstrate antibody. Unfortunately, the antibody responses in acute disease remain negative for 1 to 3 weeks after clinical onset and may never become positive in up to 20% of patients with acute, resolving disease. Current tests measure antibodies to multiple hepatitis C antigens by either enzyme immunoassay or immunoblot testing. Even with these newer assays, IgG antibody to hepatitis C may not develop for up to 4 months, making the serodiagnosis of acute hepatitis C difficult. Quantitative assays of hepatitis C RNA may be used for diagnosis, estimating prognosis, predicting interferon responsiveness, and monitoring therapy, but there is not a very good correlation between viral load and histology.

### TREATMENT AND PREVENTION

Combination therapy can benefit some persons with chronic infection

Immune globulin may not be protective; no vaccine exists

Combination therapy with interferon alpha and ribavirin is the current treatment of choice for patients with evidence of hepatitis due to hepatitis C. Criteria for initiating treatment are controversial, but most physicians would treat with abnormal liver histology and elevated liver enzymes. Responses are better in patients with genotypes other than 1 and those with low initial titers of viral RNA. Corticosteroids are not beneficial. Avoidance of injection drug use and screening of blood products are important preventive measures. It is not clear whether prophylactic ISG protects against hepatitis C. In addition, it is questionable whether a vaccine will be effective; patients may be reinfected by wild-type virus.

## *HEPATITIS E*

Hepatitis E is the cause of another form of hepatitis that is spread by the fecal–oral route and therefore resembles hepatitis A. Hepatitis E virus is an RNA virus that is similar to but distinct from caliciviruses. The viral particles in stool are spherical, 27 to 34 nm in size, and unenveloped and exhibit spikes on their surface. Like hepatitis A, infection with this virus is frequently subclinical. When symptomatic, it causes only acute disease that may fulminate, especially in pregnant women. In endemic, developing areas, it has the highest attack rate in young adults, and infection is usually associated with contaminated drinking water. It does not appear to spread from person to person. Most cases have been identified in developing countries with poor sanitation (eg, in Asia, Africa, and the Indian subcontinent), and recurrent epidemics have been described in these areas. Rarely have cases been identified in the United States, and these have been in visitors or immigrants from endemic areas. The incubation period is approximately 40 days. The diagnosis may be confirmed by demonstrating the presence of specific IgM antibody. It is likely but unproven that ISG provides protection; no treatment is available. Liver transplant may be the only recourse in seriously ill patients.

Hepatitis E spreads similarly to hepatitis A

Usually associated with contaminated drinking water

## HEPATITIS G

Although hepatitis C virus is a major cause of hepatitis, additional etiologic agent(s) continue to be sought. In 1995, hepatitis G, a newly discovered agent, was identified in sera from two different patients. Hepatitis G is an RNA virus similar to hepatitis C and members of the flavivirus family. An antibody assay can detect past, but not present, infection, and detection of acute infection with hepatitis G requires a PCR assay for viral RNA in serum. Up to 2% of volunteer blood donors are seropositive for hepatitis G RNA, which is a blood-borne virus. In addition to being closely related to hepatitis C, data suggest that the majority of patients infected by hepatitis C are also infected by hepatitis G. Given this association, it has been difficult to ascertain the contribution of hepatitis G to clinical disease. Patients infected with both viruses do not appear to have worse disease than those infected by hepatitis C virus only. Currently, there is no useful serologic test and no therapy is established.

RNA virus similar to hepatitis C

Role in human disease is currently uncertain

## ADDITIONAL READING

Defranchis R, Meucci G, Vecchi M, et al. The natural history of asymptomatic hepatitis B. *Ann Intern Med* 1993;118:191–194. A follow-up of HbsAg-positive blood donors to determine the incidence and severity of chronic hepatitis B.

Johnson Y, Lau N, Wright TL. Molecular virology and pathogenesis of hepatitis B. *Lancet* 1993;342:1335–1339. This short review covers details of molecular structure and replication of the hepatitis B virus.

Jonas MM, Kelley DA, Mizerski J, et al. Clinical trial of lamivudine in children with chronic hepatitis B. *N Engl J Med* 2002;346:1706–1713. This large collaborative study demonstrated a modest but significant benefit with 52 weeks of treatment. The commentary on pages 1682–1683 by Lok is also well worth reading.

Lauer GM, Walker BD. Hepatitis C virus infections. *N Engl J Med* 2001;345:41–52. An exceptionally well-illustrated review of the problem, including pathogenesis and treatment.

Werzberger A, et al. A controlled trial of a formalin-inactivated hepatitis A vaccine in healthy children. *N Engl J Med* 1992;327:453–457. The inactivated purified hepatitis A vaccine is well tolerated, and a single dose is highly protective against clinically apparent hepatitis A.

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# CHAPTER 38

# Herpesviruses

W. Lawrence Drew

The herpesvirus group, of the family Herpesviridae, comprises large, enveloped, double-stranded DNA viruses found in both animals and humans. They are ubiquitous and produce infections ranging from painful skin ulcers to chickenpox to encephalitis. Eight members of the family infect humans: two herpes simplex viruses (HSV-1 and HSV-2), cytomegalovirus (CMV), varicella–zoster virus (VZV), Epstein–Barr virus (EBV), human herpesvirus-6 (HHV-6), and the recently discovered human herpesvirus types 7 and 8 (HHV-7, HHV-8; Table 38–1). In addition a simian herpesvirus, herpes B virus, has occasionally caused human disease.

Large, enveloped double-stranded DNA viruses

## Group Characteristics

### VIROLOGY

All herpesviruses are morphologically similar, with an overall size of 180 to 200 nm. The DNA core is up to 75 nm in diameter and is surrounded by an icosahedral capsid. Over the capsid is a protein-filled region called the tegument. The outside of the viral particle is covered by a lipoprotein envelope derived from the nuclear membrane of the infected host cell. The envelope contains at least nine glycoproteins that protrude beyond it as spike-like structures. The viral genome is large, up to 240 kbp of DNA, which code for approximately 75 viral proteins. This large genome is necessary, because herpesviruses frequently infect nondividing cells and must therefore provide their own enzymes necessary for DNA synthesis. Despite the morphologic similarity between herpesviruses, there are substantial differences in their genomic sequences and, in turn, their structural glycoproteins and polypeptides. Antigenic analysis is an important means for differentiation among herpesviruses despite some cross-reactions (eg, between HSV and VZV).

Morphology similar among herpesviruses but genomic sequences differ

Can infect nondividing cells

Based on certain virologic similarities, the herpes viruses may be divided into three subfamilies $\alpha$, $\beta$, and $\gamma$. Herpes simplex 1 and 2, as well as varicella-zoster viruses, are in the subfamily; cytomegalovirus, HHV-6, and HHV-7 are in the $\beta$ subfamily while EBV and HHV-8 are in the $\gamma$ subfamily.

Cell tropisms for the individual viruses vary significantly. Herpes simplex virus has the widest range; it replicates in numerous animal and human host cells, although it affects only humans in nature. VZV infects only primates and is best grown in cells of human origin, although some laboratory-adapted strains can grow in primate cell lines. Human CMV replicates well only in human diploid fibroblast cell lines. EBV does not replicate in most

Herpes simplex has widest range of cell tropism

TABLE 38–1

**Human Herpesviruses**

| Designation | Common Name | Disease |
|---|---|---|
| HHV-1 | Herpes simplex virus-1 | Oral (fever blisters), ocular lesions, encephalitis |
| HHV-2 | Herpes simplex virus-2 | Genital, anal lesions<br>Severe neonatal infections, meningitis |
| HHV-3 | Varicella–zoster virus | Chickenpox (primary infection)<br>Shingles (reactivation) |
| HHV-4 | Epstein–Barr virus | Infectious mononucleosis (primary infection)<br>Tumors, including B-cell tumors (Burkitt's lymphoma, immunoblastic lymphomas of the immunosuppressed)<br>Nasopharyngeal carcinoma, some T-cell tumors |
| HHV-5 | Cytomegalovirus | Mononucleosis<br>Severe congenital infection<br>Infections in immunocompromised (gastroenteritis, retinitis, pneumonia) |
| HHV-6 | Human herpesvirus-6 | Roseola in infants (primary infection)<br>Infections in allograft recipients (pneumonia, marrow failure) |
| HHV-7 | Human herpesvirus-7 | Some cases of roseola (primary infection) |
| HHV-8 | Kaposi's sarcoma–associated herpesvirus (KSHV), human herpesvirus-8 | Tumors, including Kaposi's sarcoma<br>Some B-cell lymphomas |

commonly used cell culture systems but can be grown in continuous human or primate lymphoblastoid cell cultures. Human HSV-6 grows only in lymphocyte cell cultures.

Viral latency and disease reactivation typical for all herpesviruses

Characteristically, all of these agents produce an initial infection followed by a period of latent infection in which the genome of the virus is present in cells, but infectious virus is not recovered. During latent infection of cells, viral DNA is maintained as an episome (not integrated), with limited expression of specific virus genes required for the maintenance of latency. Reactivation of virus due to complex host–virus interactions may then result in recurrent disease. For example, immunocompromised patients, especially those with altered cellular immunity, have frequent reactivations of herpesviruses that can lead to clinically severe disease.

## Replication

Three classes of mRNAs produced

The replication of HSV is representative of all herpesviruses. The glycoproteins in the HSV envelope interact with cellular receptors to result in fusion with the cell membrane. Fusion delivers the capsid and DNA case into the cytoplasm, where it migrates to the nucleus, and the genome is circularized. Transcription of the large, complex genome is sequentially regulated in a cascade fashion. Three distinct classes of mRNAs are made: (1) immediate early (IE) mRNAs are synthesized 2 to 4 hours postinfection, which code for proteins initiating and regulating virus transcription; (2) early (E) mRNAs, which code for further nonstructural proteins involved in DNA replication and minor structural proteins; and (3) late (L) mRNAs (ie, 12 to 15 hours postinfection), which code for major structural proteins. The early (E) proteins include thymidine kinase and a DNA polymerase, which are distinct from host cell enzymes and are therefore important targets of antiviral chemotherapy. Gene expression is coordinated (ie, synthesis of early gene products turns off IE products and initiates genome replication); some of the late structural proteins are produced independently of genome replication, whereas others are only produced after replication. The pattern of viral DNA replication is complex, resulting in the formation of high-molecular-weight DNA concatemers. Genomic concatemers are cleaved and packaged into preassembled capsids in the nucleus.

Coordinated gene expression

The envelope is acquired from the inner lamella of the nuclear membrane. Budding occurs at the inner nuclear membranes, and virions then enter the cytoplasm to be released through the endoplasmic reticulum. HSV infection appears to be a "wasteful" process: only 25% of viral DNA/protein produced is incorporated into virions. The rest accumulates in the cell, which eventually dies. Moreover, the ratio of incomplete to complete viral particles is approximately 1000 to 1. Most herpesviruses shut down host cell metabolism and ultimately cause cell death, except for CMV, which actually stimulates cellular synthesis of nucleic acids and proteins.

Most herpesviruses, except CMV, shut down host cell metabolism

# HERPES SIMPLEX VIRUS

## VIROLOGY

Two distinct epidemiologic and antigenic types of HSV exist (HSV-1 and HSV-2). The DNA genomes of both are linear, double-stranded molecules containing approximately 160 kbp. Their nucleic acids demonstrate approximately 50% base sequence homology, which is considerably greater than that shown between these viruses and other herpesviruses. HSV-1 and HSV-2 share antigens in almost all their surface glycoproteins and other structural polypeptides, but differences in glycoprotein gB enable them to be distinguished (ie, HSV-1 has gB1 and HSV-2 has gB2). Numerous strains of both HSV-1 and HSV-2 exist. In fact, by restriction endonuclease analysis of the viral genome, most strains of either HSV-1 or HSV-2 are found to differ somewhat, except in epidemiologically related cases such as mother–infant and sexual partners.

HSV-1 and HSV-2 are distinct epidemiologically, antigenically, and by DNA homology

Individual strains differ by restriction endonuclease techniques

## HERPES SIMPLEX DISEASE

CLINICAL CAPSULE

HSV is one of the best known of all viruses, given its frequency of infection and its propensity to cause recurrent ulcers in areas of the skin and mucous membranes. The two types differ in their predilection for causing lesions "above the waist" (HSV-1) or "below the waist" (HSV-2). As with all herpesviruses, herpes simplex persists in a latent form and reactivates to cause viral excretion and/or disease.

### EPIDEMIOLOGY

Herpes simplex viruses are distributed worldwide. There are no known animal vectors, and humans appear to be the only natural reservoir. Direct contact with infected secretions is the principal mode of spread. Seroepidemiologic studies indicate that the prevalence of HSV antibody varies according to the age and socioeconomic status of the population studied. In most developing countries, 90% of the population have HSV-1 antibody by the

No animal reservoirs

High seroprevalence among humans, which increases with age

age of 30. In the United States, HSV-1 antibody is currently found in approximately 60 to 70% of adult middle-class populations; among lower socioeconomic groups, however, the percentage is higher.

Infection with HSV-2 linked to sexual activity

Detection of HSV-2 antibody before puberty is unusual. The virus is associated with sexual activity, and direct sexual transmission is the major mode of spread. Approximately 15 to 30% of sexually active adults in Western industrialized countries have HSV-2 antibody. The virus can be isolated from the cervix and urethra of approximately 5 to 12% of adults attending sexually transmitted disease clinics; many of these patients are asymptomatic or have small, unnoticed lesions on penile or vulvar skin. Asymptomatic shedding accounts for transmission from a partner who has no active genital lesions and often no history of genital herpes. Genital herpes is not a reportable disease in the United States, but it is estimated that more than 1,000,000 new cases occur per year.

## PATHOGENESIS

### Acute Infections

Infection produces inflammation and giant cells

Virus can infect and spread in axons and ganglia

Pathologic changes during acute infections consist of development of multinucleated giant cells (Fig 38–1), ballooning degeneration of epithelial cells, focal necrosis, eosinophilic intranuclear inclusion bodies, and an inflammatory response characterized by an initial polymorphonuclear neutrophil (PMN) infiltrate and a subsequent mononuclear cell infiltrate. The virus can spread intra- or interneuronally or through supporting cellular networks of an axon or nerve, resulting in latent infection of sensory and autonomic nerve ganglia. Spread of virus can occur by cell-to-cell transfer and can therefore be unaffected by circulating immune globulin.

### Latent Infection

No synthesis of early or late viral polypeptides in latent infection

In humans, latent infection by HSV-1 has been demonstrated by cocultivation techniques in trigeminal, superior cervical, and vagal nerve ganglia, and occasionally in the S2–S3 dorsal sensory nerve root ganglia. Latent HSV-2 infection has been demonstrated in the sacral (S2–S3) region. Latent infection of nervous tissue by HSV does not result in the death of the cell; however, the exact mechanism of viral genome interaction with the cell is incompletely understood. Several copies of the HSV viral genomes are in each latently infected neuronal cell. They exist in a circular form, and transcription of only a small portion of the viral genome occurs. Because latent infection does not appear to require synthesis of early or late viral polypeptides, antiviral drugs directed at the thymidine kinase enzymes or viral DNA polymerase do not eradicate the virus in its latent state.

Reactivation can be precipitated by sun exposure, fever, or trauma

Reactivation of virus from latently infected ganglionic cells with subsequent release of infectious virions appears to account for most recurrences of both genital and orolabial infections. The mechanisms by which latent infection is reactivated are unknown. Precipitating factors that are known to initiate reactivation of herpes simplex include; exposure to ultraviolet light, fever and trauma (eg, oral intubation).

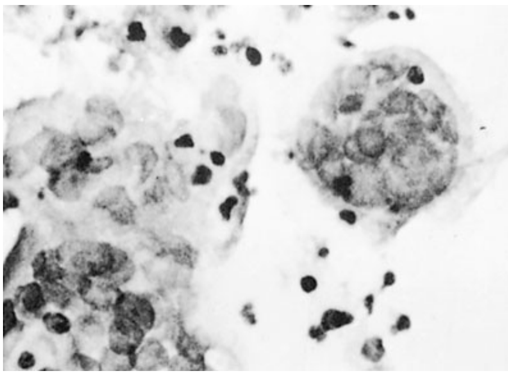

FIGURE 38–1
Multinucleated giant cells from herpes simplex virus lesion.

## IMMUNITY

Host factors have a major effect on clinical manifestations of HSV infection. Many episodes of HSV infection are either asymptomatic or mildly symptomatic. Initial symptomatic clinical episodes of the disease are more severe than recurrent episodes, probably because of the presence of anti-HSV antibodies and immune lymphocytes in persons with recurrent infections. Prior infection with HSV-1 may protect against or shorten the duration of symptoms and lesions from subsequent infection with HSV-2 due to some degree of cross protection.

Some cross-protection between HSV-1 and HSV-2

Both cellular and humoral immune responses are important in immunity to HSV. Neutralizing antibodies directed against HSV envelope glycoproteins appear to be important in preventing exogenous reinfection. Antibody-dependent cellular cytotoxicity (ADCC) may be important in limiting early spread of HSV. By the second week after infection, cytotoxic T lymphocytes can be detected that are able to destroy HSV-infected cells prior to completion of the replication cycle. Conversely, in immunosuppressed patients, especially those with depressed cell-mediated immunity, reactivation of HSV may be associated with prolonged viral excretion and persistence of lesions.

ADCC may limit early spread of HSV; cytotoxic T lymphocytes destroy HSV-infected cells

# HERPES SIMPLEX: CLINICAL ASPECTS

## MANIFESTATIONS

### Herpes Simplex Type 1

Infection with HSV-1 is usually “above the waist.” It consists characteristically of grouped or single vesicular lesions that become pustular and coalesce to form single or multiple ulcers. On dry surfaces, these ulcers scab before healing; on mucosal surfaces, they reepithelialize directly. Herpes simplex virus can be isolated from almost all ulcerative lesions, but the titer of virus decreases as the lesions evolve. Infections generally involve ectoderm (skin, mouth, conjunctiva, nervous system).

Vesicular lesions become pustular and then ulcerate

Primary infection with HSV-1 is often asymptomatic. When symptomatic, typically in children, it appears most frequently as gingivostomatitis, with fever and ulcerative lesions involving the buccal mucosa, tongue, gums, and pharynx. The lesions are quite painful, and the acute illness usually lasts 5 to 12 days. After this initial infection, HSV may become latent within sensory nerve root ganglia of the trigeminal nerve.

Primary infections often asymptomatic

Lesions usually recur on a specific area of the lip and the immediate adjacent skin; these lesions are referred to as mucocutaneous and are commonly called “cold sores” or “fever blisters.” Because reactivation is usually from a single latent source, these lesions are typically unilateral. Their recurrence may be signaled by premonitory tingling or burning in the area. Systemic complaints are unusual, and the episode generally lasts approximately 7 days. It should be noted that HSV may be reactivated and excreted into the saliva with no apparent mucosal lesions present. Herpes simplex virus has been isolated from saliva in 5 to 8% of children and 1 to 2% of adults who were asymptomatic at the time.

Recurrent cold sores usually unilateral

Virus in saliva with asymptomatic reactivation

Herpes simplex virus sometimes infects the finger or nail area. This infection, termed **herpetic whitlow,** usually results from the inoculation of infected secretions through a small cut in the skin. Painful vesicular lesions of the finger develop and pustulate; they are often mistaken for bacterial infection and mistreated accordingly.

Herpetic whitlow mimics bacterial paronychia

Herpes simplex virus infection of the eye is one of the most common causes of corneal damage and blindness in the developed world. Infections usually involve the conjunctiva and cornea, and characteristic dendritic ulcerations are produced. With recurrence of disease, there may be deeper involvement with corneal scarring. Occasionally, there may be extension into deeper structures of the eye, especially if topical steroids are used.

Herpetic corneal and conjunctival infection can cause blindness

Encephalitis may rarely result from HSV-1 infection. Most cases occur in adults with high levels of anti–HSV-1 antibody, suggesting reactivation of latent virus in the trigeminal nerve root ganglion and extension of productive (lytic) infection into the temporoparietal

Herpes encephalitis may be reactivation

area of the brain. Primary HSV infection with neurotropic spread of the virus from peripheral sites up the olfactory bulb into the brain may also result in parenchymal brain infection.

Encephalitis typically localized to temporal lobe

Rapid diagnosis allows antiviral therapy

Classically, HSV encephalitis affects one temporal lobe, leading to focal neurologic signs and cerebral edema. If untreated, mortality is 70%. Clinically, the disease can resemble brain abscess, tumor, or intracerebral hemorrhage. Rapid diagnosis by the polymerase chain reaction (PCR) has replaced brain biopsy as the diagnostic test. Intravenous acyclovir can reduce the morbidity and mortality of the disease, especially if treatment is initiated early.

## Herpes Simplex Type 2

HSV-2 associated with genital infections

Genital herpes is an important sexually transmitted disease. Both HSV-1 and HSV-2 can cause genital disease, and the symptoms and signs of acute infection are similar for both viruses. Seventy percent of first episodes of genital HSV infection in the United States are caused by HSV-2, and genital HSV-2 disease is also more likely to recur than genital HSV-1 infection. Ninety percent of HSV-2 antibody–positive patients have never had a clinically evident genital HSV episode. In many instances, the first clinical episode is years after primary infection.

### *Primary Genital Herpes Infection*

Multiple painful vesicopustular lesions

Systemic symptoms and adenopathy common

For the relatively few individuals who develop clinically evident primary genital HSV disease, the mean incubation period from sexual contact to onset of lesions is 5 days. Lesions begin as small erythematous papules that soon form vesicles and then pustules (Fig 38–2). Within 3 to 5 days, the vesiculopustular lesions break to form painful coalesced ulcers that subsequently dry; some form crusts and heal without scarring. With primary disease, the genital lesions are usually multiple (mean number, 20), bilateral, and extensive. The urethra and cervix are also infected frequently, with discrete or coalesced ulcers on the exocervix. Bilateral enlarged tender inguinal lymph nodes are usually present and may persist for weeks to months. About one third of patients show systemic symptoms such as fever, malaise, and myalgia, and approximately 1% develop aseptic meningitis with neck rigidity and severe headache. First episodes of disease last an average of 12 days.

### *Recurrent Genital Herpes Infection*

Prodromal paresthesias and shorter duration

In contrast to primary infection, recurrent genital herpes is a disease of shorter duration, usually localized in the genital region, and without systemic symptoms. A common symptom is prodromal paresthesias in the perineum, genitalia, or buttocks that occur 12 to 24 hours before the appearance of lesions. Recurrent genital herpes usually presents with grouped vesicular lesions in the external genital region. Local symptoms such as pain and itching are mild, lasting 4 to 5 days, and lesions usually last 2 to 5 days.

FIGURE 38–2
Multiple grouped vesicles of genital herpes.

At least 80% of patients with primary genital HSV-2 infection develop recurrent episodes of genital herpes within 12 months. In patients whose lesions recur, the median number of recurrences is four or five per year. They are not evenly spaced, and some patients experience a succession of monthly attacks followed by a period of quiescence. Over time, the number of recurrences decrease by a median of one-half to one recurrence per year. Most recurrences result from reactivation of virus from dorsal root ganglia. Rarely, recurrent infections may be due to reinfection with a different strain of HSV-2. Recurrent viral shedding from the genital tract may occur without clinically evident disease.

Recurrent episodes common; may involve shedding without lesions

## Neonatal Herpes

Neonatal herpes usually results from transmission of virus during delivery through infected genital secretions from the mother. In utero infection, although possible, is uncommon. In most cases, severe neonatal herpes is associated with primary infection of a seronegative woman at or near the time of delivery. This results in an intense viral exposure of a seronegative infant during the birth process. The incidence rate of neonatal herpes simplex infection varies greatly among populations, but is estimated at approximately 1 per 2500 live births in the United States. Because a normal immune response is absent in the neonate born to a mother with recent primary infection, neonatal HSV infection is an extremely severe disease with an overall mortality of approximately 60%, and neurologic sequelae are high in those who survive. Manifestations vary. Some infants show disseminated vesicular lesions with a widespread internal organ involvement and necrosis of the liver and adrenal glands, and others have involvement of the central nervous system only, with listlessness and seizures.

Usually transmitted from mother at birth

High mortality if disseminated

## DIAGNOSIS

Herpes simplex viruses are best cultured by isolation in a variety of other cell lines inoculated with infected secretions or lesions. The cytopathic effects of HSV can usually be demonstrated 24 to 48 hours after inoculation of the culture. Isolates of HSV-1 and HSV-2 can be differentiated by staining virus-infected cells with type-specific monoclonal antibodies to the two types. A direct smear prepared from the base of a suspected lesion and stained by either the Giemsa or Papanicolaou method may show intranuclear inclusions or multinucleated giant cells typical of herpes (Tzanck test), but this is less sensitive than viral culture and not specific; similar changes can be seen in cells infected with VZV. Enzyme immunoassays and immunofluorescence are rapid and relatively sensitive assays for direct detection of herpes antigen in lesions. Although early versions of these noncultural tests lacked sensitivity, more recent procedures have correlations with culture that approach 90%. Serology should not be used to diagnose active HSV infections, such as those affecting the genital or central nervous systems; frequently there is no change in antibody titer when reactivation occurs. Serology can be useful in detecting those with asymptomatic HSV-2 infection. PCR on cerebrospinal fluid (CSF) is the best test to diagnose HSV encephalitis. Restriction endonuclease digests can also be used to define epidemiologic relationships; that is, strains acquired between sexual partners or through mother–infant transmission.

Grow rapidly in many cell culture systems

HSV-1 and HSV-2 distinguished by type-specific monoclonal antibodies

Enzyme immunoassay, immunofluorescence, and PCR all used for rapid diagnosis

## TREATMENT

Several antiviral drugs that inhibit HSV have been developed. The most effective and commonly used is the nucleoside analog acyclovir, which is converted by a viral enzyme (thymidine kinase) to a monophosphate and then by cellular enzymes to the triphosphate form, which is a potent inhibitor of the viral DNA polymerase. Acyclovir significantly decreases the duration of primary infection and has a lesser but definite effect on recurrent mucocutaneous HSV infections. If taken daily, it can also suppress recurrences of genital and oral–labial HSV. In its intravenous form, it is effective in reducing mortality of HSV encephalitis and neonatal herpes. Acyclovir-resistant HSV has been recovered from immunocompromised patients with persistent lesions, especially those with acquired immunodeficiency syndrome (AIDS). Foscarnet is active against acyclovir-resistant HSV. In 1996, the U.S. Food and Drug Administration approved both valacyclovir and famciclovir for the treatment of recurrent genital HSV. Valacyclovir is a prodrug of acyclovir with better bioavailability (54% as opposed to 15–20%). It is rapidly converted to acyclovir and, in every characteristic except absorption, it is identical to the parent compound. Valacyclovir is not more effective than acyclovir but can be given in lower dose and less frequently (500 mg twice daily). Famciclovir is the prodrug of another guanosine nucleoside analog, penciclovir. The bioavailability of penciclovir is also high (77%). After conversion, penciclovir must be phosphorylated, just like acyclovir. Penciclovir has a much longer tissue half-life than acyclovir and can be given as 125 mg twice daily for treatment of recurrent genital HSV. Valacyclovir and famciclovir are now also approved for chronic suppression of recurrent genital HSV. No antiviral agents have been developed that decrease the long-term risk of subsequent reactivation of disease.

Acyclovir or prodrugs can decrease duration of acute and recurrent disease

## PREVENTION

Avoiding contact with individuals with lesions reduces the risk of spread; however, virus may be shed asymptomatically and transmitted from the saliva, urethra, and cervix by individuals with no evident lesions. Safe sex practices should reduce transmission. Although acyclovir has never been shown to reduce asymptomatic shedding from the genital tract, studies are in progress to determine whether oral antivirals can actually diminish transmission. Because of the high morbidity and mortality of neonatal infection, special attention must be paid to preventing transmission during delivery. Where active HSV lesions are present on maternal tissues, caesarean section may be used to minimize contact of the infant with infected maternal genital secretions, but caesarean section may not be effective if rupture of the membranes precedes delivery by more than several hours.

Caesarean section may be performed to avoid neonatal infection

# VARICELLA–ZOSTER VIRUS

## VIROLOGY

Varicella–zoster virus (VZV) has the same general structure as herpes simplex but contains its own envelope glycoproteins and other structures. Cellular features of infected cells such as multinucleated giant cells and intranuclear eosinophilic inclusion bodies are similar to those of HSV. VZV is more difficult to isolate in cell culture than HSV and grows best but slowly in human diploid fibroblast cells. The virus has a marked tendency to remain attached to the membrane of the host cell with less release of virions into fluids.

Slower growth and narrower range of infected cell types

## VARICELLA–ZOSTER DISEASE

**CLINICAL CAPSULE**

VZV causes two diseases, chickenpox (varicella) and shingles (zoster). The former usually occurs in children, the latter in the elderly. In the intervening years, the virus remains latent in neural ganglia but activates due to waning cellular immunity. Almost 90% of the US population is infected with VZV by the age of 10 years, and the virus is spread primarily by respiratory secretions.

## EPIDEMIOLOGY

VZV infection is ubiquitous. In temperate climates, nearly all persons contract chickenpox before they reach adulthood, and 90% of cases occur before the age of 10 years. In contrast, the mean age at infection in tropical countries is over 20 years, and the seroprevalence at age 70 may be only 50%. The virus is highly contagious, with attack rates among susceptible contacts of 75%. Varicella occurs most frequently during the winter and spring months. The incubation period is 11 to 21 days. The major mode of transmission is respiratory, although direct contact with vesicular or pustular lesions may result in transmission. Communicability is greatest 24 to 48 hours before the onset of rash and lasts 3 to 4 days into the rash. Virus is rarely isolated from crusted lesions.

Chickenpox acquired by respiratory route, usually before adulthood

Communicability greatest before rash onset

## PATHOGENESIS

Respiratory spread leads to infection of the contact patient's upper respiratory tract followed by replication in regional lymph nodes and primary viremia. The latter results in infection of the reticuloendothelial system and a subsequent secondary viremia associated with T lymphocytes. Following secondary viremia, there is infection of the skin and finally a host immune response.

Secondary viremia results in skin lesions

The relationship between zoster and varicella was first described by Von Bokay in 1892, when he observed several instances of varicella in households after the introduction of a case of zoster. On the basis of these epidemiologic observations, he proposed that zoster and varicella were different clinical manifestations of a single agent. The cultivation of VZV in vitro by Weller in 1954 confirmed Von Bokay's hypothesis: the viruses isolated from chickenpox and from zoster (or shingles) are identical. Latency of VZV occurs in sensory ganglia, as shown by in situ hybridization methods in dorsal root ganglia of adults many years after varicella infection. Herpes zoster (shingles) occurs when latent varicella zoster virus reactivates and multiplies within a sensory ganglion and then travels back down the sensory nerve to the skin. The rash of herpes zoster is generally confined to the area of the skin (ie, dermatome) innervated by the sensory ganglion in which reactivation occurs (Fig 38–3).

Varicella virus latent in sensory ganglion cells; reactivation produces zoster

## IMMUNITY

Both humoral immunity and cell-mediated immunity are important factors in determining the frequency of reinfection and reactivation of varicella–zoster. Circulating antibody prevents reinfection, and cell-mediated immunity appears to control reactivation. In patients with depressed cell-mediated immune responses, especially those with bone marrow transplants, Hodgkin's disease, AIDS, and lymphoproliferative disorders, reactivation can occur, and VZV infections are more frequent and more severe.

Circulating antibody prevents reinfection; cell-mediated immunity controls reactivation

The increase in the incidence and severity of herpes zoster observed with increasing age in immunocompetent individuals is correlated with an age-related decrease in

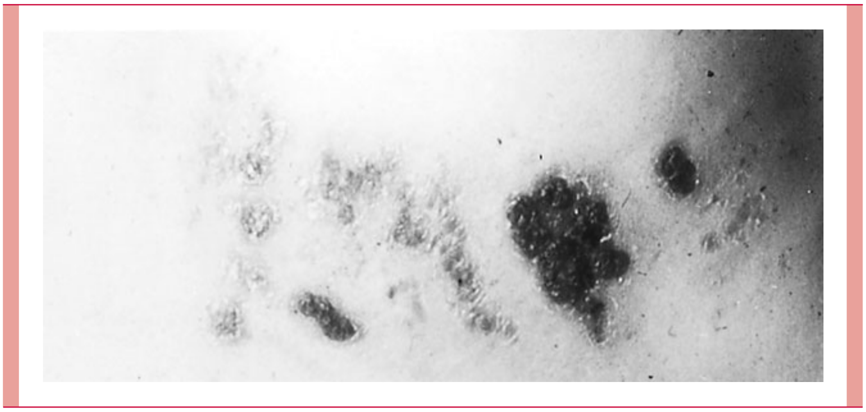

FIGURE 38–3
Herpes zoster lesion of the thorax. Note dermatomal distribution and presence of vesicles, pustules, and ulcerated and crusted lesions.

Aging associated with increasing risk of zoster

VZV-specific cellular immunity. Beginning in the fifth decade of life, there is a marked decline in cellular immunity to VZV, which can be measured by cutaneous delayed hypersensitivity as well as by a variety of in vitro assays. This occurs many years before there is any generalized decline in cellular immunity.

# VARICELLA–ZOSTER DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

Chickenpox lesions are widespread and pruritic

VZV produces a primary infection in normal children characterized by a generalized vesicular rash termed **chickenpox** or **varicella.** After clinical infection resolves, the virus persists for decades in the absence of clinical manifestation. Chickenpox lesions generally appear on the back of the head and ears, then spread centrifugally to the face, neck, trunk, and proximal extremities. Involvement of mucous membranes is common, and fever may occur early in the course of disease. Lesions appear in different stages of evolution; this characteristic is one of the major features used to differentiate varicella from smallpox, in which lesions are concentrated on the extremities and all had a similar appearance. Varicella lesions are pruritic (itchy), and the number of lesions may vary from 10 to several hundred.

Severe disease in immunocompromised patients

Immunocompromised children may develop progressive varicella, which is associated with prolonged viremia and visceral dissemination as well as pneumonia, encephalitis, hepatitis, and nephritis. Progressive varicella has an estimated mortality of approximately 20%. In thrombocytopenic patients, the lesions may be hemorrhagic. Susceptible adults are at higher risk (15×) for VZV pneumonia during chickenpox.

Reactivation to zoster most common in elderly

Follow sensory nerve distribution

Reactivation of VZV is associated with the disease herpes zoster (shingles). Although zoster is seen in patients of all ages, it increases in frequency with advancing age. Clinically, pain in a sensory nerve distribution may herald the onset of the eruption, which occurs several days to a week or two later. The vesicular eruption is usually unilateral, involving one to three dermatomes. New lesions may appear over the first 5 to 7 days. Multiple attacks of VZV infection are uncommon; if recurrent attacks of a vesicular eruption occur in one area of the body, HSV infection should be considered.

Postherpetic neuralgia after zoster

Dissemination with visceral infection in immunocompromised persons

The complications of VZV infection are varied and depend on age and host immune factors. Postherpetic neuralgia is a common complication of herpes zoster in elderly adults. It is characterized by persistence of pain in the dermatome for months to years after resolution of the lesions of zoster and appears to result from damage to the involved nerve root. Immunosuppressed patients may develop localized zoster followed by dissemination of virus with visceral infection, which resembles progressive varicella. Bacterial superinfection is also possible. Maternal varicella infection during early pregnancy can result in fetal embryopathy with skin scarring, limb hypoplasia, microcephaly, cataracts, chorioretinitis, and microphthalmia. Severe varicella can also occur in seronegative neonates, with mortality as high as 30%.

## DIAGNOSIS

Diagnosis usually clinical

Rapid confirmation by immunofluorescent staining

Varicella or herpes zoster lesions can be diagnosed clinically, although they may occasionally be difficult to distinguish from those caused by HSV or even vaccinia smallpox. Scrapings of lesions may reveal multinucleated giant cells characteristic of herpesviruses, but cytologic examination does not distinguish HSV lesions from those due to VZV. For rapid viral diagnosis, the best procedure is to demonstrate varicella–zoster antigen in cells from lesions by immunofluorescent antibody staining. VZV can be isolated from vesicular fluid or cells inoculated onto human diploid fibroblasts; however, the virus is difficult to grow from zoster (shingles) lesions older than 5 days, and cytopathic effects are usually not seen for 5 to 9 days. PCR of CSF may be useful in the diagnosis of VZV encephalitis; culture is rarely positive.

## TREATMENT

Acyclovir has been shown to reduce fever and skin lesions in patients with varicella, and its use is recommended in healthy patients over 18 years of age. There are insufficient data to justify universal treatment of all healthy children and teenagers with varicella. In immunosuppressed patients, controlled trials of acyclovir have been effective in reducing dissemination, and the use of this agent is definitely indicated. In addition, controlled trials of acyclovir have demonstrated effectiveness in the treatment of herpes zoster in immunocompromised patients. Acyclovir may be used to treat herpes zoster in immunocompetent adults, but it appears to have only a modest impact on the development of post-herpetic neuralgia, the most important complication of zoster. Treatment should be started within 3 days of the onset of zoster. VZV is less susceptible than HSV to acyclovir, so the dosage for treatment is substantially higher. Famciclovir or valacyclovir are more convenient and may be more effective.

Acyclovir or related prodrug therapy of immunocompromised patients

## PREVENTION

High-titer immune globulin administered within 96 hours of exposure is useful in preventing infection or ameliorating disease in patients at risk for severe primary infection (eg, immunosuppressed children who are household or play contacts of patients with varicella or zoster). Once skin lesions have occurred, high-titer immune globulin has not proved useful in ameliorating disease or preventing dissemination. Immune globulin is not indicated for the treatment or prevention of reactivation (ie, zoster or shingles). In nonimmunosuppressed children, varicella is a relatively mild disease, and passive immunization is not indicated.

Passive immunization for immunocompromised

A live vaccine developed by a group of Japanese workers appears to be effective in both immunosuppressed and immunocompetent persons and is now recommended for routine use after 12 months of age in healthy children (Table 38–2). In immunocompromised patients who are susceptible to varicella, chickenpox can be extremely serious, even fatal. In these patients, the live vaccine appears to be protective although it is not approved for this use in the United States. The vaccine is being used routinely in immunocompetent seronegative adults, especially those at occupational risk, such as health care workers, and it can even be helpful if given to a seronegative, immunocompetent adult shortly after exposure. Varicella is a highly contagious disease, and rigid isolation precautions must be instituted in all hospitalized cases.

Live vaccine is safe and effective

Need for isolation of cases in hospital

TABLE 38–2

### Properties of the Live Attenuated Varicella Vaccine (Oka)

- Rarely causes rash (5% in healthy children, mild)
- One dose induces antibody, which persists for >10 years in >90% of healthy children. Two doses are required for adults
- Induces cell-mediated immunity
- Lack of contact infection in most cases
- Induces long-term protective immunity
- Prevents disease when administered up to 3 days after exposure (postexposure prophylaxis)
- Incidence of herpes zoster in vaccinated children with leukemia is lower than in comparable children infected naturally with wild-type virus
- >90% protection vs. household exposure of healthy children

# CYTOMEGALOVIRUS

## VIROLOGY

Nuclear and perinuclear cytoplasmic inclusions and cell enlargement

Human cytomegalovirus (CMV) possesses the largest genome of the herpesviruses (~240 kbp), and its replication, although slow, is similar to HSV with the sequential appearance of immediate early, early and late gene products. In addition to nuclear inclusions ("owl eye cells"), CMV produces perinuclear cytoplasmic inclusions and enlargement of the cell (cytomegaly), a property that gives the virus its name. Based on genomic and phenotypic heterogeneity, innumerable strains of CMV exist, and restriction endonuclease analysis of viral DNA has been useful for distinguishing strains epidemiologically. Antigenic variations have been observed but are not of clinical importance.

## CYTOMEGALOVIRUS DISEASE

CLINICAL CAPSULE

CMV differs from HSV and VZV by not causing skin disease, but CMV is similar in its ability to establish latent infection. CMV produces visceral disease, including a mononucleosis syndrome in otherwise healthy individuals. Its major contribution to human misery is a high rate of congenital infection (1% of all infants; 40,000 in the United States per year), most of whom are asymptomatic; however, some 20% may have neurologic impairment. CMV is also an important cause of morbidity and mortality in immunocompromised patients with either primary or reactivation disease.

### EPIDEMIOLOGY

High infection rates in early childhood and early adulthood

Present in urine, saliva, semen, and cervical secretions

CMV is ubiquitous, and in developed countries approximately 50% of adults have developed antibody. Age-specific prevalence rates show that approximately 10 to 15% of children are infected by CMV during the first 5 years of life, after which the rate of new infections levels off. The rate subsequently increases by 1 to 2% per year during adulthood, probably through close personal contact, including sexual, with a virus-excreting person. CMV has been isolated from saliva, cervical secretions, semen, urine, and white blood cells for months to years following infection. Excretion of CMV is especially prolonged after congenital and perinatal infections, with 35% of infected infants excreting virus for as long as 5 years after birth. Transmission of infection in day-care centers has been shown to occur from asymptomatic excreters to other children and, in turn, to seronegative parents. By age 18 months, up to 80% of infants in a day-care center are infected and actively excreting virus in saliva and urine. Seroconversion rates in seronegative parents who have children attending day-care centers are approximately 20% per year. This increases to approximately 30% if the child is shedding virus and up to 40% if the child is also under 18 months of age. In contrast to day-care centers, there is no substantial evidence of spread of CMV infection to health care workers in the hospital.

Viral latency in leukocytes

Latent infection, which occurs in leukocytes and their precursors, accounts for transfusion transmission, but this route is relatively infrequent; only 1 to 2% of blood units are believed to be infectious. Organ donation may also transmit latent virus, which causes primary infection in CMV-seronegative recipients and reinfection in seropositive patients.

### PATHOGENESIS

As previously mentioned, CMV infects epithelial cells and leukocytes and produces characteristic inclusions in the former. In vitro, CMV DNA can be demonstrated in monocytes showing no cytopathology, indicating a restricted growth potential in these cells. It is conjectured that these are the cells of latency for CMV.

CMV DNA in monocytes

CMV can cause disease by a variety of different mechanisms, including direct tissue damage and immunologic damage. While direct infection and damage of mucosal epithelial cells in the lung is a potential mechanism for pneumonia, animal models have suggested that immunologic destruction of the lung by the host immune response to CMV infection may be the major mechanism of viral disease in this tissue. This hypothesis is supported by the observation that the degree of viral infection in lung tissue cannot account for the severity of CMV pneumonia; likewise, the disease does not respond well to antiviral therapy. While cytolytic T-lymphocyte activity may contribute to lung pathology, cytokines released by these cells have also been implicated.

Immune-mediated tissue damage

## IMMUNITY

Both humoral and cellular immune responses are important in CMV infections. In immunocompetent persons, clinical disease, if it occurs at all, results from primary infection, and reactivation with viral excretion in cervical excretions or semen is invariably subclinical. In immunocompromised patients, both primary infection and reactivation are much more likely to be symptomatic. Furthermore, CMV infection of monocytes results in dysfunction of these phagocytes in immunocompromised patients, which may increase predisposition to fungal and bacterial superinfection. When latently infected monocytes are in contact with activated T lymphocytes, the former are activated to differentiate into macrophages that produce infectious virus. These monocyte–T cell interactions may occur following transfusion or transplantation and may explain not only transmission of CMV but also activation of latent virus in the allograft recipient. Vascular endothelial cells may be other sites of CMV latency.

Vascular endothelial cells can be infected and support viral latency

# CYTOMEGALOVIRUS: CLINICAL ASPECTS

## MANIFESTATIONS

Worldwide, 1% of infants excrete CMV in urine or nasopharynx at delivery as a result of infection in utero. On physical examination, 90% of these infants appear normal or asymptomatic; however, long-term follow-up has indicated that 10 to 20% go on to develop sensory nerve hearing loss, psychomotor mental retardation, or both. Infants with symptomatic illness (about 0.1% of all births) have a variety of congenital defects or other disorders, such as hepatosplenomegaly, jaundice, anemia, thrombocytopenia, low birth weight, microcephaly, and chorioretinitis. Almost all infants with clinically evident congenital CMV infection are born of mothers who experienced primary CMV infection during pregnancy. The apparent explanation is that these babies are exposed to virus in the absence of maternal antibody. It is estimated that one third of maternal primary infections are transmitted to the fetus and that fetal damage is most likely to occur in the first trimester. Congenital infection frequently also results from reactivation in the mother with spread to the fetus, but such infection rarely leads to congenital abnormalities since the mother also transmits antibody to the fetus.

Serious disease of fetus may develop with primary maternal infection

In contrast to the devastating findings with some congenital infections, neonatal infection acquired during or shortly after birth appears to be rarely associated with an adverse outcome. Most population-based studies have indicated that 10 to 15% of all mothers are excreting CMV from the cervix at delivery. Approximately one third to one half of all infants born to these mothers acquire infection. Almost all of these perinatally infected infants have no discernible illness unless the infant is premature or immunocompromised. CMV can also be efficiently transmitted from mother to child by breast milk, but these postpartum infections are also usually benign.

Perinatal infection asymptomatic or relatively benign

CMV pneumonia, visceral, and eye infections in immunocompromised patients

As with intrapartum acquisition of infection, most CMV infections during childhood and adulthood are totally asymptomatic. In healthy young adults, CMV may cause a mononucleosis-like syndrome. In immunosuppressed patients, both primary infection and reactivation may be severe. For example, in patients receiving bone marrow transplants, interstitial pneumonia caused by CMV is a leading cause of death (50–90% mortality) and in AIDS patients, CMV often disseminates to visceral organs, causing chorioretinitis, gastroenteritis, and neurologic disorders.

## DIAGNOSIS

DNA detection by PCR or antigen detection useful to find viremia

Laboratory diagnosis of CMV infection depends on (1) detecting CMV cytopathology, antigen, or DNA in infected tissues; (2) isolating the virus from tissue or secretions; or (3) demonstrating seroconversion. CMV can be grown readily in serially propagated diploid fibroblast cell lines. Demonstration of cytopathic effect generally requires 3 to 14 days, depending on the concentration of virus in the specimen and whether coverslip cultures in shell vials are used to speed detection. The presence of large inclusion-bearing cells in urine sediment may be detected in widespread CMV infection. This technique is insensitive, however, and provides positive results only when large quantities of virus are present in the urine. Culture of blood to detect viremia is now superseded by detection of CMV antigen in peripheral blood leukocytes or detection of CMV DNA in plasma or leukocytes. These procedures are more rapid and more sensitive than culture.

Histologic detection of inclusions in lung, gastrointestinal tissues is useful

Because of the high prevalence of asymptomatic carriers and the known tendency of CMV to persist weeks or months in infected individuals, it is frequently difficult to associate a specific disease entity with the isolation of the virus from a peripheral site. Thus, the isolation of CMV from urine of immunosuppressed patients with interstitial pneumonia does not constitute evidence of CMV as the cause of that illness. CMV pneumonia or gastrointestinal disease is best diagnosed by demonstrating CMV inclusions in biopsy tissue.

The procedures listed below are recommended to facilitate the diagnosis of CMV infection in specific clinical settings:

1. Congenital infection. Virus culture or viral DNA assay positive at birth or within 1 to 2 weeks (to distinguish from natally or perinatally infected infants, who will not begin to excrete virus until 3 to 4 weeks after delivery).
2. Perinatal infection. Culture-negative specimens at birth but positive specimens at 4 weeks or more after birth suggest natal or early postnatal acquisition. Seronegative infants may acquire CMV from exogenous sources, e.g. blood transfusion.
3. CMV mononucleosis in nonimmunocompromised patients. Seroconversion and presence of IgM antibody specific for CMV are the best indicators of primary infection. Urine culture positivity supports the diagnosis of CMV infection but may reflect remote infection, because positivity may continue for months to years. A positive blood assay for CMV antigen or DNA, however, is diagnostic in this patient population.
4. Immunocompromised patients. Demonstration of virus by viral antigen, DNA, or culture in blood documents viremia. Demonstration of inclusions or viral antigen in diseased tissue (eg, lung, esophagus, or colon) establishes the presence of CMV infection but does not provide proof that CMV is the cause of disease unless other pathogens are excluded. Seroconversion is diagnostic but rarely occurs, especially in AIDS patients, because more than 95% of these patients are seropositive for CMV before infection with human immunodeficiency virus (HIV). CMV-specific IgM antibody may not be present in immunocompromised patients, especially during reactivation of virus. Conversely, in AIDS patients, this antibody frequently is present even when clinically important infection is absent.

## TREATMENT

Ganciclovir, a nucleoside analog of acyclovir, has been shown to inhibit CMV replication; prevent CMV disease in AIDS patients and transplant recipients; and reduce the severity of some CMV syndromes, such as retinitis and gastrointestinal disease. Combining immune globulin with ganciclovir appears to reduce the very high mortality of CMV pneumonia in bone marrow transplant patients over that achieved with ganciclovir alone, but the prognosis for long-term survival of these patients remains poor. Foscarnet, a second approved drug for therapy of CMV disease, is equally efficacious. Its toxic effects are primarily renal, whereas ganciclovir is most apt to inhibit bone marrow function. Ganciclovir inhibits CMV DNA polymerase, like foscarnet, but the two drugs act on different sites, and cross resistance is rare. In 1996, a third drug, cidofovir, a nucleotide analog, was approved for therapy of retinitis; it is also nephrotoxic.

Ganciclovir used with immune globulin

## PREVENTION

The use of blood from CMV-seronegative donors or blood that is treated to remove white cells decreases transfusion-associated CMV. Similarly, the disease can be avoided in seronegative transplant recipients by using organs from CMV-seronegative donors. Hyperimmune human anti-CMV globulin has been used to ameliorate CMV pneumonia associated with transplants. Safe sex practices including condom usage reduces transmission. CMV vaccines have been developed, and are being evaluated in clinical trials.

Use of CMV seronegative donors decreases risk

# EPSTEIN–BARR VIRUS

## VIROLOGY

Epstein–Barr virus (EBV) is the etiologic agent of infectious mononucleosis and African Burkitt's lymphoma. Its complete nucleotide sequence of 172 kbp is smaller than other herpes viruses but has been thoroughly mapped. Although EBV is morphologically similar to the other herpesviruses, it can be cultured easily only in lymphoblastoid cell lines derived from B lymphocytes of humans and higher primates. In vivo, EBV is tropic for both human B lymphocytes and epithelial cells. The former is a nonproductive infection, while the latter is productive. The virus generally does not produce cytopathic effects or the characteristic intranuclear inclusions of other herpesvirus infections. After infection with EBV, lymphoblastoid cells containing viral genome can be cultivated continuously in vitro; they are thus transformed, or immortalized. Recent studies suggest that most of the viral DNA in transformed cells remains in a circular, nonintegrated form as an episome, while a lesser amount is integrated into the host cell genome. Viral antigen expression has been studied by immunofluorescent staining of transformed cell lines under various conditions. One group of proteins, called EBV nuclear antigens (EBNAs), appear in the nucleus prior to virus-directed protein synthesis. Viral capsid antigen (VCA) can be detected in cell lines that produce mature virions. Other cell lines, called nonproducers, contain no mature virions, but express certain virus-associated antigens called early antigens (EAs). The latter may be seen as diffuse (D) and as restricted (R) aggregates of staining.

Etiologic agent of infectious mononucleosis and certain lymphomas

Cultivated only in lymphoblastoid cell lines

EBNA, VCA, and EA represent stages of viral replication

## EPSTEIN–BARR VIRUS DISEASE

CLINICAL CAPSULE

Investigators discovered EBV in the course of their studies to determine the cause of Burkitt's lymphoma. Serologic studies later found that the virus was the cause of infectious mononucleosis. The greatest interest in EBV hinges on its role in malignant disease, including Burkitt's lymphoma, nasopharyngeal carcinoma, and lymphoproliferative disease of the immunocompromised.

## EPIDEMIOLOGY

Widespread asymptomatic infection; disease most common in young adults

EBV can be cultured from saliva of 10 to 20% of healthy adults and is intermittently recovered from most seropositive individuals. It is of low contagiousness, and most cases of infectious mononucleosis are contracted after repeated contact between susceptible persons and those asymptomatically shedding the virus. Secondary attack rates of infectious mononucleosis are low (<10%), because most family or household contacts already have antibody to the agent (worldwide 90–95% of adults are seropositive). Infectious mononucleosis has also been transmitted by blood transfusions; most transfusion-associated mononucleosis syndromes, however, are attributable to CMV. In more highly developed countries and in individuals of higher socioeconomic status, EBV infection tends to be acquired later in life than in individuals from developing countries of lower socioeconomic status. When primary infection with EBV is delayed until the second decade of life or later, it is accompanied by symptoms of infectious mononucleosis in about 50% of cases.

At present, there appears to be many fewer variations of genomic strains among EBV isolates than other herpesviruses.

## PATHOGENESIS

Infects B cells

Encodes proteins associated with immortalization of B cells

Although EBV initially infects epithelial cells, the hallmark of EBV disease is subsequent infection of B lymphocytes and polyclonal B lymphocyte activation with benign proliferation. The virus enters B lymphocytes by means of envelope glycoprotein binding to a surface receptor CD21, which is the receptor for the C36 component of complement; 18 to 24 hours later, EBV nuclear antigens are detectable within the nucleus of infected cells. Expression of the viral genome, which encodes at least two viral proteins, is associated with immortalization and proliferation of the cell. The EBV-infected B lymphocytes are polyclonally activated to produce immunoglobulin and express a lymphocyte-determined membrane antigen that is the target of host cellular immune responses to EBV-infected B lymphocytes. During the acute phase of infectious mononucleosis, up to 20% of circulating B lymphocytes demonstrate EBV antigens. After infection subsides, EBV can be isolated from only about 1% of such cells.

Lymphomas can develop in immunocompromised patients

EBV has been associated with several lymphoproliferative diseases, including African Burkitt's lymphoma, nasopharyngeal carcinoma, and lymphomas in immunocompromised patients. The factors that render the EBV infections oncogenic in these cases are obscure. The distribution of EBV infections in Africa has suggested an infectious cofactor, such as malaria, which may cause immunosuppression and predispose to EBV-related malignancy. In nasopharyngeal carcinoma, environmental carcinogens may create the precancerous lesion although genetic factors may also be operative. In vivo, EBV-associated lymphomas have been shown to be of both monoclonal and polyclonal origin. Chromosomal translocations in B cells are characteristic of Burkitt's lymphoma and involve specific breaks in chromosomes. These translocations lead to expression of oncogenes that may contribute to clonal activation and ultimately to malignancy. Some breakdown in immune surveillance also appears to play a role in the development of malignancy, because immunosuppressed patients are more prone to develop EBV associated B-cell lymphomas.

## IMMUNITY

Suppressed cell-mediated immune responses in acute infection

Virus-induced infectious mononucleosis is associated with circulating antibodies against specific viral antigens, as well as against unrelated antigens found in sheep, horse, and some beef red blood cells. The latter, referred to as heterophile antibodies, are a heterogeneous group of predominantly IgM antibodies long known to correlate with episodes of infectious mononucleosis, and are commonly used as diagnostic tests for the disease. They do not cross-react with antibodies specific for EBV, and there is not good correlation between the heterophile antibody titer and the severity of illness. Cutaneous anergy and decreased cellular immune responses to mitogens and antigens are seen early in the course of mononucleosis. The "atypical" lymphocytosis associated with infectious mononucleosis is caused by an increase in the number of circulating T cells, which appear to be activated cells developed in response to the virus-infected B lymphocytes. With recovery from illness, the atypical lymphocytosis gradually resolves, and cell-mediated immune functions return to preinfection levels, although memory T cells maintain the capacity to limit proliferation of EBV-infected B cells. In rare cases, the initial EBV-induced proliferation of B cells is not contained, and EBV lymphoproliferative disease ensues. This syndrome is most often seen in immunocompromised organ transplant recipients.

# EPSTEIN–BARR VIRUS: CLINICAL ASPECTS

## MANIFESTATIONS

### Infectious Mononucleosis

Although most primary EBV infections are asymptomatic, clinically apparent infectious mononucleosis is characterized by fever, malaise, pharyngitis, tender lymphadenitis, and splenomegaly. These symptoms persist for days to weeks; they slowly resolve. Complications such as laryngeal obstruction, meningitis, encephalitis, hemolytic anemia, thrombocytopenia, or splenic rupture may occur in 1 to 5% of patients.

Primary infection asymptomatic or expressed as infectious mononucleosis

### Lymphoproliferative Syndrome

Patients with primary or secondary immunodeficiency are susceptible to EBV-induced lymphoproliferative disease. For example, the incidence of these lymphomas is 1 to 2% following renal transplants and 5 to 9% following heart–lung transplants. The risk is greatest in patients experiencing primary EBV infection rather than reactivation. Most characteristic is persistent fever, lymphadenopathy, and hepatosplenopathy.

Lymphoproliferative disease occurs, especially in immunocompromised persons

### Burkitt's Lymphoma

In sub-Saharan Africa, Burkitt's lymphoma is the most common malignancy in young children, with an incidence of 8 to 10 cases per 100,000 people per year. The risk is greatest in equatorial Africa, where there is a high incidence of malaria. Burkitt's lymphoma is thought to result from an early EBV infection that produces a large pool of infected B lymphocytes. Malarial infection may further increase the size of this pool and provide a constant antigenic challenge. Serologic screening for increased IgA antibody levels to both VCA and early EBV antigens can be used for early diagnostic purposes.

Tumors may involve cofactors

Translocation may lead to clonal activation

### Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) is endemic in southern China, where it is responsible for approximately 25% of the mortality from cancer. The high incidence of NPC among the southern Chinese people suggests that genetic or environmental factors in addition to EBV may also be important in the pathogenesis of the disease.

Endemic NPC in southern China; suggests environmental or genetic cofactors

### AIDS Patients

In AIDS patients, several distinct additional EBV-associated diseases may occur, including hairy leukoplakia of the tongue, interstitial lymphocytic pneumonia (especially in infants), and lymphoma.

## DIAGNOSIS

Laboratory analysis of EBV infectious mononucleosis is usually documented by the demonstration of atypical lymphocytes, and heterophile antibodies, or positive EBV-specific serologic findings. Hematologic examination reveals a markedly raised lymphocyte and monocyte count with more than 10% atypical lymphocytes, called Downey cells (Fig 38–4). Atypical lymphocytes, although not specific for EBV, are present with the onset of symptoms and disappear with resolution of disease. Alterations in liver function tests may also occur, and enlargement of the liver and spleen is a frequent finding.

Atypical lymphocytosis common in acute infection

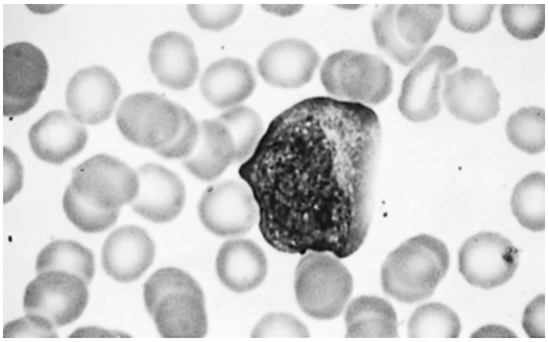

FIGURE 38–4
Atypical lymphocyte (Downey cell) in blood smear from a patient with infectious mononucleosis. Note indented cell membrane.

Heterophile antibodies nonspecific but appear early

Although not specific for EBV, tests for heterophile antibodies are used most commonly for diagnosis of infectious mononucleosis. In commercial kits, animal erythrocytes are used in simple slide agglutination methods, which incorporate absorptions to remove cross-reacting antibodies that may develop in other illnesses, such as serum sickness. The infectious mononucleosis heterophile antibody is absorbed by sheep erythrocytes but not by a guinea pig kidney cells. Heterophile antibodies can usually be demonstrated by the end of the first week of illness but may occasionally be delayed until the third or fourth week. They may persist many months.

IgM antibody to VCA suggest acute, primary infection

Approximately 5 to 15% of EBV-induced cases of infectious mononucleosis in adults and a much greater proportion in young children and infants fail to induce detectable levels of heterophile antibodies. In these cases, the EBV-specific serologic tests summarized in Table 38–3 may be used to establish the diagnosis. The panel to be tested includes antibodies to VCA, which rise quickly and persist for life. Antibodies to EBNAs rise later in

TABLE 38–3
**Epstein–Barr Virus-Specific Antibodies**

| Antibody Specificity | Time of Appearance in Infectious Mononucleosis | Duration | Comments |
|---|---|---|---|
| Viral capsid antigen (VCA) | | | |
| IgM | Early in illness | 1–2 months | Indicator of primary infection |
| IgG | Early in illness | Lifelong | Standard EBV titer reported by most commercial and state labs; major utility is as a marker for prior infection in epidemiologic studies; if present in the absence of EBNA antibody, indicates current infection |
| EBNA IgG | 3–6 weeks after onset | Lifelong | Late appearance of anti-EBNA IgG antibodies in IM makes absence or seroconversion a useful marker for primary infection; persists for life |
| Early antigen | | | |
| EA diffuse protein (EA-D) | Peaks 3–4 weeks after onset | 3–6 months | Present in IM patients; IgA antibodies useful for prediction of NPC in high-risk populations |
| EA restricted (EA-R) | Several weeks after onset | Months to years | Present in higher titer in African Burkitt's lymphoma; may be useful as indicator of reactivation of EBV |

*Abbreviations*: EA, early antigen; EBV, Epstein–Barr virus; IM, infectious mononucleosis; NPC, nasopharyngeal carcinoma; EBNA, EBV nuclear antigen.

disease (after about 1 month) and also persist in low titers for life. Thus, a high titer to VCA and no titer to EBNA suggests recent EBV infection, whereas antibody titers to both antigens are indicative of past infection. The presence of IgM antibody to VCA is theoretically diagnostic of acute, primary EBV infection, but low levels may occur during reactivation of EBV and cross-reactions with antigens of other herpesviruses occur. Persistent antibody to early antigens (anti-EA, -D, or -R) may be correlated with severe disease, nasopharyngeal carcinoma (anti-D), or African Burkitt's lymphoma (anti-R), but are not useful in diagnosing infectious mononucleosis. Isolation of EBV from clinical specimens is not practical, because it requires fresh human B cells or fetal lymphocytes obtained from cord blood.

Virus isolation is impractical for routine diagnosis

## TREATMENT

Treatment of infectious mononucleosis is largely supportive. More than 95% of patients recover uneventfully. In a small percentage of patients, splenic rupture may occur; restriction of contact sports or heavy lifting during the acute illness is recommended. The DNA polymerase enzyme of EBV has been shown to be sensitive to acyclovir, and acyclovir can decrease the amount of replication of EBV in tissue culture and in vivo. Despite this antiviral activity, systemic acyclovir makes little or no impact on the clinical illness. Laryngeal obstruction should be treated with corticosteroids. Hairy leukoplakia in AIDS patients does respond to acyclovir treatment.

Treatment is supportive

## PREVENTION

The occurrence of Burkitt's lymphoma and nasopharyngeal carcinoma in restricted geographic areas offers the possibility of prevention by immunization with virus-specific antigen(s). At present, this approach is under exploration. A subunit vaccine has proved effective in preventing the development of tumors in tamarind monkeys, which are highly susceptible to the oncogenic effects of the virus under experimental conditions.

Immunization of humans not available

# *HUMAN HERPESVIRUS-6*

In 1986, a herpesvirus, now called human herpesvirus type-6 (HHV-6), was identified in cultures of peripheral blood lymphocytes from patients with lymphoproliferative diseases. The virus, which is genetically distinct but morphologically similar to other herpesviruses, replicates in lymphoid tissue, especially CD4+ T lymphocytes and has two distinct variants, A and B. HHV-6 is more closely related to CMV than to the other earlier known herpesviruses and is the $\beta$ subfamily.

Replicates in CD4+ T lymphocytes

## EPIDEMIOLOGY

HHV-6 is the most rapidly spread of the herpesviruses and is shed in the throats of 10% of babies by age 5 months, 70% by 12 months, and 30% of adults. Almost all of the population has antibody to this virus by the age of 5 years.

Infection common in infancy

## MANIFESTATIONS

HHV-6 type B is the etiologic agent of exanthem subitum (roseola), and both types A and B can cause acute febrile illnesses with or without seizures or rashes. Exanthem subitum generally occurs in infants aged 6 months to 1 year. It is characterized by fever (usually about 39ºC) for 3 days, followed by a faint maculopapular rash spreading from the trunk to the extremities, which begins during defervescence.

Associated with roseola in infants

HHV-6 also appears to reactivate in transplant recipients. It may contribute to graft rejection and clinical illnesses such as meningoencephalitis, pneumonia, and bone marrow suppression after bone marrow transplantation. The virus reactivates in other

Reactivation common in immunosuppression immunocompromised patients including those with AIDS, lymphoma, and leukemia, but its clinical significance is not known.

Latent infection of T cells

Initially, it was thought that HHV-6 would grow only in freshly isolated B lymphocytes, and the virus was referred to as the human B lymphotropic virus. Now it is clear that the virus infects mainly T lymphocytes. HHV-6 establishes a latent infection in T cells but may be activated to a productive lytic infection by mitogenic stimulation. Resting lymphocytes and lymphocytes from normal immune individuals are resistant to HHV-6 infection. In vivo, HHV-6 replication is controlled by cell-mediated factors.

## DIAGNOSIS

Primary infection can be documented serologically

PCR used to detect viremic infection

Primary virus infection can be documented by seroconversion. Active virus infection can be documented by culture, antigenemia, or DNA detection in the blood (by PCR). Because asymptomatic viremic reactivation is common, it is very difficult to use these tools to identify HHV-6 as the cause of febrile or other miscellaneous syndromes.

## TREATMENT

No viral thymidine kinase

Definitive therapy has not been established, but HHV-6 appears to be susceptible in vitro to ganciclovir and foscarnet. It is less susceptible to acyclovir, because the virus has no thymidine kinase.

# HUMAN HERPESVIRUS-7

Originally isolated from CD4+ T lymphocytes

Can cause exanthem subitum (roseola)

Isolation of human herpesvirus-7 (HHV-7) was first reported in 1990. The virus was isolated from activated CD4+ T lymphocytes of a healthy individual. The CD4 molecule appears to be a receptor for virus attachment. HHV-7 is distinct from all other known human herpesviruses but is most closely related to HHV-6 and CMV and is in the $\beta$ subfamily with these two viruses. Seroepidemiologic studies indicate that this virus usually does not infect children until after infancy but that nearly 90% of children are antibody positive by 3 years of age. As with HHV-6, this virus is frequently isolated from saliva, and close personal contact is the probable means of transmission. Also, like HHV-6, this virus may be a cause of exanthem subitum. The diagnosis of acute infection can be made by the demonstration of seroconversion. No treatment has been identified.

# HUMAN HERPESVIRUS-8

Human herpesvirus-8 (Kaposi's sarcoma–associated herpesvirus, or KSHV; HHV-8) was discovered in 1994 by identification of unique viral DNA sequences in Kaposi's sarcoma tissue obtained from an AIDS patient, using subtractive hybridization analysis. These specific DNA sequences are found in 95% or more of Kaposi's sarcoma tissues, both AIDS related and non-AIDS in African cases. KSHV DNA has also been detected in cells from lymphoproliferative diseases (eg, primary effusion lymphomas, associated with AIDS and multicentric Castleman's disease).

Associated with Kaposi's sarcoma

Recently, HHV-8 was isolated in culture, and when characterized, it seems most closely related to EBV. Like EBV, the virus preferentially infects B lymphocytes and it is also considered to be a gamma herpes virus. Epidemiologic and virologic studies suggest that it is a necessary but perhaps not sufficient cause of Kaposi's sarcoma and that other factors (eg, immunosuppression, genetic predisposition) are cofactors in the development of this malignancy. On average, seropositivity to HHV-8 precedes the development of

Kaposi's sarcoma by 3 years. The virus appears to be sexually transmitted, as suggested by a higher prevalence of antibody in promiscuous gay men than those who are not promiscuous, and by higher prevalence in gay men with HIV versus other HIV-positive risk groups, such as transfusion recipients and hemophiliacs. Specific and sensitive antibody assays are being developed, and antibody to HHV-8 appears to be relatively rare in the general population. It is difficult to assess the impact of antivirals, because Kaposi's sarcoma may improve with immune reconstitution. Interferon-$\alpha$ can be effective against Kaposi's sarcoma, but this may result from immune enhancement rather than any specific antiviral activity. Evidence of active viral replication in Kaposi's sarcoma is minimal, so there may not be an appropriate target for antivirals at the time that Kaposi's sarcoma becomes manifest.

Infects B lymphocytes

## ADDITIONAL READING


### Herpes Simplex Virus

Brown ZA, Selke S, Zeh J, et al. The acquisition of herpes simplex virus during pregnancy. *N Engl J Med* 1997;337:509–515. Acquisition of infection with seroconversion completed before labor does not appear to affect the outcome of pregnancy, but infection acquired near the time of labor is associated with an increased risk of severe neonatal herpes infection.

Wald A. Herpes. Transmission and viral shedding. *Dermatol Clin* 1998;16:795–797. Reviews data on asymptomatic shedding of HSV from the genital tract of HSV-2 antibody–positive patients and its impact on transmission.

Wald A, Carrell D, Remington M, et al. Two day regimen of acyclovir for treatment of recurrent genital herpes simplex virus type 2 infection. *Clin Infect Dis* 2002;34:944–948. Reviews current therapy of genital HSV infection and introduces an effective 2-day acyclovir treatment option.

Whitley RJ, Kimberlin DW, Roizman B. Herpes simplex viruses. *Clin Infect Dis* 1998;26:541–555. This is a well-referenced, thorough review of HSV-1 and HSV-2.

### Varicella–Zoster Virus

Gnann JW Jr, Whitley RJ. Herpes zoster. *N Engl J Med* 2002;347:340–346. A well-illustrated, excellent review, including current management guidelines and antiviral and other treatments to prevent postherpetic neuralgia.

### Cytomegalovirus

Boppana SB, Rivera LB, Fowler KB, et al. Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. *N Engl J Med* 2001;344:1366–1371. This article illustrates how reinfection during pregnancy with different CMV strains can lead to intrauterine transmission and also includes an excellent review of the literature.

Drew WL. Ganciclovir resistance: Matter of time and titre. *Lancet* 2000;356:609–610. An editorial review of CMV resistance in transplant recipients and the lessons learned in HIV-positive patients.

Nichols WG, Corey L, Gooley T, et al. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)–seronegative recipients of stem cell transplants from seropositive donors: Evidence for indirect effects of primary CMV infection. *J Infect Dis* 2002;185:273–282. An excellent background review of the role of CMV in immunocompromised patients and analysis of the significant immunomodulatory effects of CMV infection in predisposing to serious nonviral disease.

Paya CV, Wilson JA, Espy MJ, et al. Preemptive use of oral ganciclovir to prevent cytomegalovirus infection in liver transplant patients: A randomized placebo-controlled trial.

*J Infect Dis* 2002;185:854–860. Outlines past and present approaches to detection of CMV viremia and preemptive treatment.

## Epstein–Barr Virus

Cohen JI. Epstein–Barr virus infection. *N Engl J Med* 2000;343:481–492. Excellent review, with illustrations and reference.

Mitarnum W, Suwiwat S, Pradutkanchana J, et al. Epstein–Barr virus–associated peripheral T-cell and NK-cell proliferative disease/lymphoma: Clinicopathologic, serologic, and molecular analysis. *Am J Hematol* 2002;70:31–38. A comprehensive review of 100 patients with EBV-associated lymphoproliferative disease. Presents evidence for active replication of EBV in lymphocytes.

## Human Herpesvirus-6

Caserta MT, Mock DJ, Dewhurst S. Human herpesvirus 6. *Clin Infect Dis* 2001;33: 829–833. This is a concise, well-referenced review.

Hall CB, Long CE, Schnabel KC, et al. Human herpesvirus-6 infections in children: A prospective study of complications and reactivation. *N Engl J Med* 1994;331:432–438. This report identifies the contribution of HHV-6 to febrile disease, seizures, and rash.

## Human Herpesvirus-7

Caserta MT, Hall CB, Schnabel K, et al. Primary human herpesvirus 7 infection: A comparison of human herpesvirus 7 and human herpesvirus 6 infections in children. *J Pediatr* 1998;133:386–389. This paper covers the clinical and laboratory diagnostic issues encountered.

## Human Herpesvirus-8

Ablashi DV, Chatlynne LG, Whitman JE Jr., Cesarman E. Spectrum of Kaposi's sarcoma-associated herpesvirus, or human herpesvirus 8, diseases. *Clin Microbiol Rev* 2002;15:439–464. Extensive review of the biology of KSHV and its potential for producing disease.

CHAPTER 39

# Viruses of Diarrhea

C. George Ray

Acute diarrheal disease is an illness, usually of rapid evolution (within several hours), that lasts less than 3 weeks. In addition to the bacterial and protozoal agents responsible for approximately 20 to 25% of these cases, viruses are a significant cause of the balance. Rotaviruses, caliciviruses, astroviruses, and some adenoviruses are considered here. Unfortunately, investigations have been hampered because most of these viruses cannot be readily cultivated in the laboratory.

## General Features

Until the 1970s, proof of viral causation of acute diarrhea was usually based on exclusion of known bacterial or protozoan pathogens and supported by feeding cell-free filtrates of diarrheal stools to volunteers in an attempt to reproduce the disease. As might be expected, the results of such experiments were variable, and the methods were impractical for routine laboratory diagnosis. One aspect of such infections that proved of great help was the frequent association with abundant excretion of virus particles during the acute phase of illness. Virion numbers in excess of $10^8$ per gram of diarrheal stool are relatively common, allowing ready visualization with an electron microscope. Direct electron microscopy and immunoelectron microscopy have been frequently used to detect and identify the presumed causative viruses; the latter method can also be used to detect humoral antibody responses to infection. More recently, polymerase chain reactions (PCR) and enzyme immunoassays (EIA) have been increasingly used for diagnosis.

Viral diarrhea was a diagnosis of exclusion

Many viral particles seen in stool by electron microscopy

Confirmation by EIA or PCR is now possible

Detection of a specific virus in the stools of symptomatic patients is not sufficient to establish the role of the virus in causing disease. Other criteria to be fulfilled include the following: (1) establish that the virus is detected in ill patients significantly more frequently than in asymptomatic, appropriately matched controls and that virus shedding temporally correlates with symptoms; (2) demonstrate significant humoral or secretory antibody responses, or both, in patients shedding the virus; (3) reproduce the disease by experimental inoculation of nonimmune human or animal hosts (usually the most difficult criterion to fulfill); (4) exclude other known causes of diarrhea, such as bacteria, bacterial toxins, and protozoa. Using these criteria, four groups of viruses have been clearly established as important causes of gastrointestinal disease: rotaviruses, caliciviruses, astroviruses, and some adenovirus serotypes ("enteric" adenoviruses). Other viruses have also been implicated, but all of the preceding criteria have not been

Multiple criteria used for establishing etiologic relationship

Rotaviruses, caliciviruses, astroviruses, and adenoviruses are established

"Candidate" viruses meet some criteria

fulfilled; therefore, they are currently regarded as "candidate" causes of gastrointestinal disease.

Vomiting commonly follows short incubation period

The currently established viruses are listed in Table 39–1 and all have several features in common, including a tendency toward brief incubation periods; fecal–oral spread by direct or indirect routes; and production of vomiting, which generally precedes or accompanies the diarrhea. The last feature has influenced physicians to use the term **acute viral gastroenteritis** to describe the syndrome associated with these agents.

## ROTAVIRUSES

Most common cause of winter gastroenteritis in children <2 years of age

The human intestinal rotaviruses were first found in 1973 by electron microscopic examination of duodenal biopsy specimens from infants with diarrhea. Since then, they have been found worldwide and are believed to account for 40 to 60% of cases of acute gastroenteritis occurring during the cooler months in infants and children less than

TABLE 39–1

**Biological and Epidemiologic Characteristics of Viruses that Cause Diarrhea**

| SPECIAL FEATURES | ROTAVIRUS | CALICIVIRUS | ASTROVIRUS | ADENOVIRUS |
|---|---|---|---|---|
| **BIOLOGICAL** | | | | |
| Nucleic acid | Double-stranded RNA | Single-stranded RNA | Single-stranded RNA | Double-stranded DNA |
| Diameter, shape | 65–75 nm, naked, double-shelled capsid | 27–38 nm, naked, round | 28–38 nm, naked, star-shaped | 70–90 nm, naked, icosahedral |
| Replication in cell culture | Usually incomplete | None | None | None or incomplete |
| Number of serotypes | 4 important to humans | More than 4 | 5, perhaps more | Unknown |
| **PATHOGENIC** | | | | |
| Site of infection | Duodenum, jejunum | Jejunum | Small intestine | Small intestine |
| Mechanism of immunity | Local intestinal IgA | Unknown | Unknown | Unknown |
| **EPIDEMIOLOGIC** | | | | |
| Epidemicity | Epidemic or sporadic | Family and community outbreaks | Sporadic | Sporadic |
| Seasonality | Usually winter | None known | None known | None known |
| Ages primarily affected | Infants, children <2 y old | Older children and adults | Infants, children | Infants, children |
| Method of tr1ansmission | Fecal–oral | Fecal–oral; contaminated water and shellfish | Fecal–oral | Fecal–oral |
| Incubation period (days) | 1–3 | 0.5–2 | ?1–2 | 8–10 |
| Major diagnostic tests | EIA, EM[a] | EM, IEM, PCR | EM, PCR | EIA, EM |

[a] *Abbreviations:* EM, electron microscopy; IEM, immunoelectron microscopy; EIA, enzyme immunoassay; PCR, polymerase chain reaction.

2 years of age. These viruses have been detected in intestinal contents and in tissues from the upper gastrointestinal tract.

## VIROLOGY

The rotaviruses belong to the family Reoviridae. They are naked, spherical particles 65 to 75 nm in diameter (smaller forms have also been described) with a genome containing 11 segments of double-stranded RNA and a double-shelled outer capsid; two segments encode proteins of the outer capsid (VP4 and VP7), which are targets for neutralizing antibodies. The name is derived from the Latin **rota** ("wheel") because of the outer capsid, which resembles a wheel attached by short spokes to the inner capsid and core (Fig 39–1). Three serogroups have been associated with disease in humans (groups A, B, and C). Four group A serotypes (1, 2, 3, and 4), based on VP7 type-specific antigens on the outer capsid, are of major epidemiologic importance. Rotaviruses can replicate in the cytoplasm of infected cell cultures in the laboratory but are difficult to propagate because the replicative cycle is usually incomplete, and mature, infectious virions are often not produced. However, successful propagation of human strains in vitro has been achieved in some instances.

Double-stranded RNA viruses are shaped like a wheel

Antigenic types are based on capsid proteins VP4 and VP7

Rotaviruses of animal origin are also highly prevalent and produce acute gastrointestinal disease in a variety of species. Very young animals, such as calves, suckling mice, piglets, and foals, are particularly susceptible. The animal rotaviruses can often replicate in cell cultures, and infection across species lines has been accomplished experimentally; however, there is no evidence that such interspecies spread occurs in nature (eg, animal rotaviruses are not known to affect humans and vice versa).

Animal rotaviruses produce diarrhea but interspecies spread not demonstrated in nature

One unique feature of rotaviruses is the ease with which the 11 RNA segments can undergo reassortment. This has enabled the development of live vaccines that combine genes from readily cultivated animal rotaviruses with human rotavirus genes that encode serotype-specific capsid proteins. For example, a current vaccine combines 10 RNA segments from a naturally attenuated rhesus monkey serotype 3 rotavirus with one human rotavirus genomic segment that encodes serotype 1, 2, and 4 VP7 neutralization specificities.

Reassortment of the 11 RNA segments readily occurs

Live vaccine accounts for variation

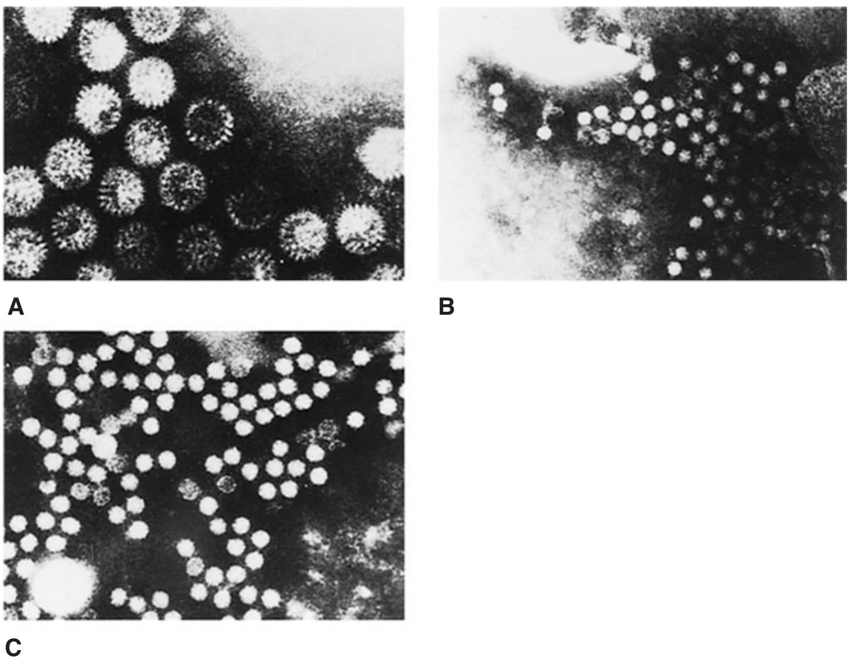


FIGURE 39–1
Viruses of diarrhea. **A.** Rotavirus. **B.** Calicivirus. **C.** Astrovirus. (*Courtesy of Claire M. Payne.*)

# HUMAN ROTAVIRUS INFECTIONS

CLINICAL CAPSULE

Worldwide, an estimated one million infants die each year as a result of rotavirus diarrhea. Currently, in the United States, the total annual deaths now are thought to be less than 100, but these viruses are still major causes of severe illness and hospitalization in early life. Vomiting, abdominal cramps, and low-grade fever, followed by watery stools that usually do not contain mucus, blood, or pus, are all characteristic of the acute phase of illness, and can also be seen with infections due to caliciviruses, astroviruses and adenoviruses.

## EPIDEMIOLOGY

Primarily infants and children in colder months

Outbreaks of rotavirus infection are common, particularly during the cooler months, among infants and children 1 to 24 months of age. Older children and adults can also be affected, but attack rates are usually much lower. Outbreaks among elderly, institutionalized patients have also been recognized.

Most older children and adults are immune

Although newborn infants can be readily infected with the virus, such infections often result in little or no clinical illness. This finding is illustrated by reported infection rates of 32 to 49% in some neonatal nurseries, but mild illness in only 8 to 28% of the infants. It is unclear whether this transient resistance to disease is a result of host maturation factors or transplacentally conferred immunity. Seroepidemiologic studies have been useful in demonstrating the ubiquity of these viruses and, perhaps, help to explain the age-specific attack rates. By the age of 4 years, more than 90% of individuals have humoral antibodies, suggesting a high rate of virus infection early in life.

## PATHOGENESIS

Destroys villus cells of jejunum and duodenum

Absorptive surface is decreased

Enterotoxin-like effects are also present

Rotaviruses appear to localize primarily in the duodenum and proximal jejunum, causing destruction of villous epithelial cells with blunting (shortening) of villi and variable, usually mild, infiltrates of mononuclear and a few polymorphonuclear inflammatory cells within the villi. The gastric and colonic mucosa are unaffected; however, for unknown reasons, gastric emptying time is markedly delayed. The primary pathophysiologic effects are a decrease in absorptive surface in the small intestine and decreased production of brush border enzymes, such as the disaccharidases. The net result is a transient malabsorptive state, with defective handling of fats and sugars. It may take as long as 3 to 8 weeks to restore the normal histologic and functional integrity of the damaged mucosa. While the specific gene product associated with virulence is not yet known, some evidence suggests that one nonstructural protein, NSP4, may behave as an enterotoxin in a manner similar to the heat-labile enterotoxin (LT) of *Escherichia coli* and cholera toxin. This may further explain the excess fluid and electrolyte secretion in the acute phase of illness. Viral excretion usually lasts 2 to 12 days but can be greatly prolonged in malnourished or immunodeficient patients, with persistent symptoms.

## IMMUNITY

Type-specific humoral and secretory IgA antibodies are protective

IgA and mucin glycoproteins confer protective role of breastfeeding

Patients with rotavirus infection respond with production of type-specific humoral antibodies that appear to last for years, perhaps a lifetime. In addition, type-specific secretory IgA (sIgA) antibodies are produced in the intestinal tract, and their presence seems to correlate best with immunity to reinfection. Breastfeeding also seems to play a protective role against rotavirus disease in young infants. Secretory IgA antibodies to rotaviruses appear in colostrum and continue to be secreted in breast milk for several months postpartum. Human breast milk mucin glycoproteins have also been shown to bind to rotaviruses, inhibiting their replication in vitro and in vivo.

## ROTAVIRUS INFECTIONS: CLINICAL ASPECTS

### MANIFESTATIONS

After an incubation period of 1 to 3 days, there is usually an abrupt onset of vomiting, followed within hours by frequent, copious, watery, brown stools. In severe cases, the stools may become clear; the Japanese refer to the disease as **hakuri,** the "white stool diarrhea." Fever, usually low grade, is often present. Vomiting may persist for 1 to 3 days, and diarrhea for 4 to 8 days. The major complications result from severe dehydration, occasionally associated with hypernatremia.

This complication can lead to death, particularly in very small or malnourished infants

Short incubation period, vomiting, and watery diarrhea can lead to dehydration

### DIAGNOSIS

Diagnosis of acute rotavirus infection is usually by detection of virus particles or antigen in the stools during the acute phase of illness. This can be accomplished by direct examination of the specimen by electron microscopy or, more conveniently, by immunologic detection of antigen with EIA methods (see Chapter 15).

Electron microscope or EIA detect virus

### TREATMENT AND PREVENTION

There is no specific treatment. Vigorous replacement of fluids and electrolytes is required in severe cases and can be life-saving. The rotaviruses are highly infectious and can spread quickly in family and institutional settings. Control consists of rigorous hygienic measures, including careful hand washing and adequate disposal of enteric excretions. Live attenuated reassortant vaccines have been developed, as noted previously. The findings to date indicate that such an approach to control or amelioration of the natural infection is feasible. However, there remains some concern about safety, particularly with regard to reports of an increased risk of intussusception among recently immunized infants. Until this issue is resolved, vaccine will not be made available for routine use.

Live, attenuated, or recombinant vaccines are feasible

Intussuseption is vaccine safety concern

## CALICIVIRUSES

Although the caliciviruses were the first to be clearly associated with outbreaks of gastroenteritis, considerably less is known about their biology than about that of the rotaviruses. They were first associated with an outbreak in Norwalk, Ohio, in 1968, and their role was confirmed by production of disease in volunteers fed fecal filtrates. The original virus was thus called the **Norwalk agent,** and similar viruses have been given names such as Hawaii agent, Montgomery County agent, Ditchling agent, and so on.

## VIROLOGY

The viruses are small, naked, round RNA-containing particles 27 to 38 nm in diameter; their appearance is similar to that of the DNA-containing parvoviruses and hepatitis A virus (see Fig 39–1). They are classified as members of the Caliciviridae family. At present, two genera that cause diarrhea are recognized within this family: "Norwalk-like viruses" (sometimes referred to as "Noroviruses") and "Sapporo-like viruses." The viruses appear to be extremely hardy; their infectivity persists after exposure to acid, ether, and heat (60°C for 30 minutes). They have not been effectively propagated in cell or organ culture.

Small, round unenveloped RNA viruses are hardy

Two genera: "Norwalk-like" and "Sapporo-like"

At least four different serotypes have been demonstrated by immunoelectron microscopy with convalescent sera from affected patients. Knowledge of the antigenic

Several serotypes but not yet grown

characteristics and biology of these viruses has been seriously hampered by the current inability to grow them in the laboratory and by their lack of known pathogenicity for animals.

## CALICIVIRUS INFECTIONS

### EPIDEMIOLOGY

Sharp outbreaks include older children and adults

Transmission is by fecal–oral route

Sharp family and community outbreaks are common and can occur in any season. Unlike rotaviruses, caliciviruses are much more common causes of gastrointestinal illness in older children and adults. This difference in age-specific predilection is perhaps reflected in serosurveys, which have shown that the prevalence of antibodies rises slowly, reaching approximately 50% by the fifth decade of life, a striking contrast to the frequent acquisition of antibodies to rotaviruses early in life. Transmission is primarily fecal–oral; outbreaks have also been associated with consumption of contaminated water, uncooked shellfish, and other foods.

### PATHOGENESIS

Enterotoxic features are not present

Both the pathogenesis and the pathology are similar to those described for rotaviruses, except that no enterotoxic features have yet been described for caliciviruses. The mucosal changes usually revert to normal within 2 weeks of onset of illness. Virus shedding in the feces generally lasts no more than 3 to 4 days.

### IMMUNITY

Reinfection can occur with same serotypes

Patients and experimentally infected volunteers respond to infection with the production of humoral antibodies, which persist indefinitely; their role in protection from reinfection, however, appears minimal. Reinfection and illness with the same serotype occur, and the role of local antibody has not been well defined. It is possible that nonimmune or genetic factors are essential for protection.

## CALICIVIRUS INFECTIONS: CLINICAL ASPECTS

Clinical picture and diagnostic tests are similar to rotavirus

No treatment or vaccine exists

The incubation period is 10 to 51 hours, followed by abrupt onset of vomiting and diarrhea, a syndrome clinically indistinguishable from that caused by rotaviruses. Respiratory symptoms rarely coexist, and the duration of illness is relatively brief (usually 1–2 days). These viruses can be detected by electron microscopy or immunoelectron microscopy in stools during the acute phase of illness. In addition, EIA and PCR methods have been developed. As with rotavirus infection, there is no specific treatment other than fluid and electrolyte replacement. Prevention requires good hygienic measures.

## ADENOVIRUSES, ASTROVIRUSES, AND "CANDIDATE" VIRUSES

Serotypes 40 and 41 are commonly found

Some **adenoviruses,** most of which are exceedingly difficult to cultivate in vitro (in contrast to those associated with respiratory diseases), are now recognized as significant intestinal pathogens. They may account for an estimated 5 to 15% of all viral gastroenteritis in young children. These include serotypes 40, 41, and perhaps 38.

**Astroviruses** have a shape that resembles a 5- or 6-pointed star (see Fig. 39–1). These have been known since 1975. In recent years astroviruses have been acknowledged as causes of often mild gastroenteritis outbreaks, primarily among toddlers, school children and elderly nursing home residents.

Illness is often, but not always, mild

Other agents associated with gastrointestinal diseases include coronavirus-like agents, toroviruses, and some group A coxsackieviruses (the latter primarily cause gastrointestinal symptoms in severely immunocompromised patients). This list may grow in the future; however, until more is learned about their biology, epidemiologic behavior, and impact on human health, they remain "candidate" viruses for now.

## ADDITIONAL READING

Glass RI, Gentsch JR, Ivanoff B. New lessons for rotavirus vaccines. *Science* 1996; 272:46–48. This brief review provides excellent insight into the biology and importance of rotaviruses.

Kapikian AZ. Viral gastroenteritis. *JAMA* 1993;269:627–630. This prominent investigator presents a concise, well-referenced overview of the relative importance of these agents.

Lundgren O, Peregrin AT, Persson K, et al. Role of the enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhea. *Science* 2000;287:491–495. This report and the accompanying commentary on pp. 409–411 show how basic research can provide leads to new therapeutic approaches.

Murphy TV, Gargiullo PM, Massoudi MS, et al. Intussusception among infants given an oral rotavirus vaccine. *N Engl J Med* 2001;344:564–572. This large investigation well illustrates the reasons why close surveillance for vaccine-associated adverse events are so important.

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CHAPTER 40

# Arthropod-Borne and Other Zoonotic Viruses

C. George Ray

The zoonotic viruses comprise more than 400 agents, one or more of which occur in most parts of the world. Members of the group have their ultimate reservoirs in lower vertebrates or insects. They are from diverse taxonomic families of RNA viruses that primarily include the togaviruses, bunyaviruses, reoviruses, arenaviruses, and filoviruses. Their major morphologic and genetic features are summarized in Table 5–1. Certain DNA viruses (poxviruses) are also transmissible from animals to humans. These are considered in Chapter 35.

The zoonotic viruses discussed here are divided into two groups. The arboviruses are transmitted to humans by infected bloodsucking insects such as mosquitoes, ticks, and *Phlebotomus* flies (sandflies). The other zoonotic RNA viruses are generally believed to be transmitted by inhalation of infected animal excretions, by the conjunctival route, or occasionally by direct contact with infected animals. Rabies virus, which is commonly transmitted by animal bites, is discussed separately in Chapter 41.

## VIROLOGY

In most cases, the zoonotic viruses were first named after the place of initial isolation (eg, St. Louis encephalitis) or after the disease produced (eg, yellow fever). More recent studies have assigned the majority to families and genera on the basis of properties indicated in Table 5–1. The major characteristics of these families are summarized below.

Generally named after place of isolation

### TOGAVIRUSES AND FLAVIVIRUSES

Togaviruses and flaviviruses are enveloped virions containing single-stranded, positive-sense RNA measuring 40 to 70 nm in external diameter. The envelope contains a hemagglutinin and lipoproteins. Virions mature by budding from cellular membranes. Replication can occur in cells of infected arthropods and vertebrate hosts. The *Alphavirus* and *Flavivirus* genera within these families include most arthropod-borne viruses. Each genus possesses its own unique primary structure of the RNA genome. Viruses within these genera are frequently serologically related to one another but not to others. Representatives are listed in Table 40–1.

Enveloped RNA viruses contain hemagglutinin and lipoproteins

TABLE 40–1

**Selected Arboviruses of Major Importance to Humans**

| Genus and Member | Major Geographic Distribution | Primary Arthropod Vector | Usual Disease Expression |
|---|---|---|---|
| **Togaviruses** | | | |
| *Alphavirus* | | | |
| Western equine encephalitis | North America | Mosquito | Encephalitis |
| Eastern equine encephalitis | North America | Mosquito | Encephalitis |
| Venezuelan equine encephalitis | Central and South America | Mosquito | Encephalitis |
| Chikungunya | Africa and Asia | Mosquito | Febrile illness |
| **Flaviviruses** | | | |
| *Flavivirus* | | | |
| St. Louis encephalitis | North America | Mosquito | Encephalitis |
| Dengue | All tropical zones | Mosquito | Febrile illness or hemorrhagic fever |
| Yellow fever | Africa, South America, and Caribbean | Mosquito | Hepatic necrosis, hemorrhage |
| West Nile fever | Africa, Eastern Europe, Middle East, Asia, North America | Mosquito | Febrile illness or encephalitis |
| Murray Valley encephalitis | Australia | Mosquito | Encephalitis |
| Russian spring–summer encephalitis | Eastern Soviet Union and Central Europe | Tick | Encephalitis |
| Powassan | Canada | Tick | Encephalitis |
| Japanese B encephalitis | Japan, Korea, and Philippines | Mosquito | Encephalitis |
| **Bunyaviruses** | | | |
| *Bunyavirus* | | | |
| California | North America | Mosquito | Encephalitis |
| Bunyamwera | Africa | Mosquito | Febrile illness |
| Rift Valley fever | Africa | Mosquito | Febrile illness |
| Sandfly fever | Mediterranean | *Phlebotomus* | Febrile illness |
| **Reoviruses** | | | |
| *Orbivirus* | | | |
| Colorado tick fever | North America | Tick | Febrile illness |

## BUNYAVIRUSES

Spherical, enveloped RNA viruses mature by budding

Bunyaviruses are spherical, enveloped, single-stranded negative-sense RNA viruses approximately 90 to 100 nm in external diameter. They mature by budding into smooth-surfaced vesicles in or near the Golgi region of the infected cell. The major disease-causing bunyaviruses in North America are California virus and hantavirus.

## REOVIRUSES

Unenveloped RNA viruses are prominent in North America

Reoviruses are spherical, unenveloped, double-stranded RNA viruses that measure about 80 nm in diameter with a segmented genome. The most important North American arbovirus of this family, which is a member of the genus *Coltivirus,* causes Colorado tick fever.

## ARENAVIRUSES

The arenaviruses are enveloped, spherical or pleomorphic viruses containing single-stranded, negative-sense RNA in several segments and measuring 50 to 300 nm in diameter. They mature by budding from host cell cytoplasmic membranes and contain host cell ribosomes in their interior. These ribosomes confer a granular appearance to the viruses, hence their name (from the Latin **arenosus** for "sandy"). The most significant arenavirus infections in humans are the hemorrhagic fevers, including Lassa fever. The virus of lymphatic choriomeningitis is occasionally transmitted to humans from infected mice and other rodents.

Spherical, enveloped RNA contain host cell ribosomes

## FILOVIRUSES

Filoviruses are enveloped, single-stranded, negative-sense RNA viruses. They are filamentous and highly pleomorphic, averaging 80 nm in diameter and 300 to 14,000 nm in length as they bud from the cell membrane. They are the cause of Marburg and Ebola fevers, two highly fatal hemorrhagic fevers.

Enveloped filamentous RNA viruses cause hemorrhagic fevers

# ARBOVIRUSES

## ARBOVIRUS DISEASE

CLINICAL CAPSULE

Some arboviruses cause severe inflammation of the brain (encephalitis) with damage or destruction of neural cells that may be fatal or lead to permanent neurologic damage in survivors. Others, such as dengue viruses, can produce illnesses that range from mild flu-like symptoms to overwhelming shock with widespread hemorrhage into tissues. Still another, yellow fever virus, primarily attacks liver cells, leading to extensive destruction and sometimes fatal liver failure.

## EPIDEMIOLOGY

Arboviruses of major importance in human disease are listed in Table 40–1 with summaries of their geographic distribution, the arthropod vectors that transmit them, and the usual disease syndromes that can result from infection.

With the exception of urban dengue and urban yellow fever, in which the virus may simply be transmitted between humans and mosquitoes, other arboviral diseases involve nonhuman vertebrates. These are usually small mammals, birds, or, in the case of jungle yellow fever, monkeys. Infection is transmitted within the host species by arthropods (eg, mosquitoes or ticks) that become infected. In some cases, the infection can be maintained from generation to generation in the arthropod by transovarial transmission. Infection in the arthropod usually does not appear to harm the insect; however, a period of virus multiplication (termed **extrinsic incubation period**) is required to enhance the capacity to transmit infection to vertebrates by bite. The consequences of infection transmitted from the arthropod to susceptible vertebrate hosts are variable; some develop illness of varying severity with viremia, whereas others may have long-term viremia without clinical disease. Vertebrate hosts are then a source of further spread of the virus by amplification, in which noninfected arthropods feeding on viremic hosts acquire the virus, thereby increasing the risk of transmission.

Reservoirs are in nonhuman vertebrates

Sometimes maintained by vertical transmission in vector

Multiplication in vector is required

Transient viremia is a feature of many of these infections in hosts other than their reservoir; those affected, including humans and higher vertebrates (eg, horses and cattle), Sustained viremia required for vertebrate host to be significant reservoir

are often referred to as blind-end hosts. In contrast, if viremia is sustained for longer periods (eg, weeks to months in a variety of togavirus, flavivirus, and bunyavirus infections of lower vertebrates), the vertebrate host becomes highly important as a reservoir for continuing transmission. Viremia may last a week or more in human dengue and yellow fever infections, and humans may then serve as a reservoir in urban disease.

Season-to-season survival has multiple mechanisms

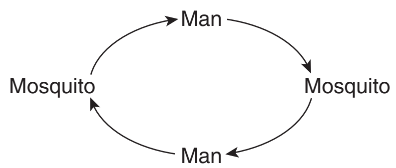
Obviously, the usual arthropod vectors are rarely present during all seasons. The question then arises as to how the arboviruses survive between the time the vector disappears and the time it reappears in subsequent years. Several mechanisms can operate to sustain the virus between transmission periods (often referred to as **overwintering**): (1) sustained viremia in lower vertebrates such as small mammals, birds, and snakes, from which newly mature arthropods can be infected when taking a blood meal; (2) hibernation of infected adult arthropods that survive from one season to the next; and (3) transovarial transmission, whereby the infected female arthropod can transmit virus to its progeny.

The three basic cycles of arbovirus transmission are urban, sylvatic, and arthropod-sustained.

## Urban

Urban cycle exists with dengue and yellow fever

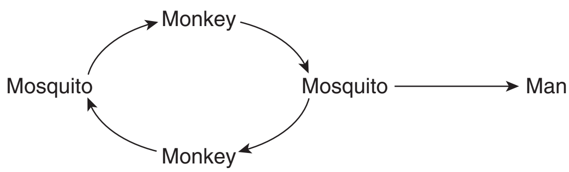
As the term suggests, the urban cycle is favored by the presence of relatively large numbers of humans living in close proximity to arthropod (usually mosquito) species capable of virus transmission. The cycle is:




Examples of this cycle include urban dengue, urban yellow fever, and occasional urban outbreaks of St. Louis encephalitis.

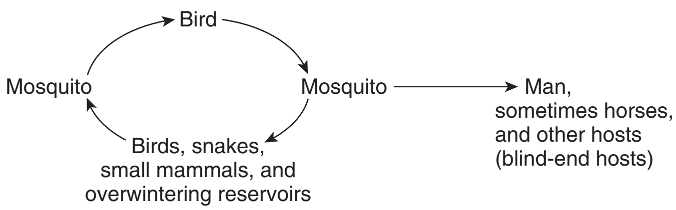
## Sylvatic

In the sylvatic cycle a single nonhuman vertebrate reservoir may be involved:




In this situation, the human, who becomes a tangential host through accidental intrusion into a zoonotic transmission cycle, is not important in maintaining the infection cycle. An example of this cycle is jungle yellow fever.

In other sylvatic cycles, multiple vertebrate reservoirs may be involved:



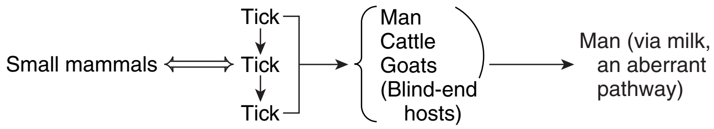

Sylvatic cycle occurs with many viruses

Humans are tangential hosts

Examples include western equine encephalitis, eastern equine encephalitis, and California viruses. In some situations, such as St. Louis encephalitis and yellow fever, the urban and sylvatic cycles may operate concurrently.

## Arthropod-Sustained

Arthropods, especially ticks, may sustain the reservoir by transovarial transmission of virus to their progeny, with amplification of the cycle by spread to and from small mammals:




Arthropod sustained by tick transovarial transmission

Weather, swamps, and ponds alter conditions

Tick-borne encephalitis in Russia is transmitted by this cycle. In temperate climates such as the United States, arboviruses are major causes of disease during the summer and early fall months, the season of greatest activity of arthropod vectors (usually mosquitoes or ticks). When climatic conditions and ecologic circumstances (eg, swamps and ponds) are optimal for arthropod breeding and egg hatching, arbovirus amplification may begin.

Mosquito increases create risk for blind-end human infection

An example of amplification is provided by western equine encephalitis. When the mosquito vectors become abundant, the level of transmission among the basic reservoir hosts (birds and small mammals) increases, and the mosquitoes also turn to other susceptible species such as the domestic fowl. These hosts experience a rapidly developing asymptomatic viremia, which permits still more arthropods to become infected on biting. At this point, spread to blind-end hosts such as humans or horses and the development of clinical disease become likely. This occurrence depends on the accessibility of the host to the infected mosquito and on mosquito feeding preferences which, for unknown reasons, vary from one season to another.

## PATHOGENESIS

CNS, visceral, and hemorrhagic fever are major syndromes

There are three major manifestations of arbovirus diseases in humans associated with different tropisms of various viruses for human organs, although overlap can occur. In some, the central nervous system (CNS) is primarily affected, leading to aseptic meningitis or meningoencephalitis. A second syndrome involves many major organ systems, with particular damage to the liver, as in yellow fever. The third is manifested by hemorrhagic fever, in which damage is particularly severe to the small blood vessels, with skin petechiae and intestinal and other hemorrhages.

After bite, viremia and viral tissue tropism define disease

In CNS, aseptic meningitis and encephalitis follow cell injury

Infection of the human by a biting, infected arthropod is followed by viremia, which is apparently amplified by extensive virus replication in the reticuloendothelial system and vascular endothelium. After replication the virus becomes localized in various target organs, depending on its tropism, and illness results. The viruses produce cell necrosis with resultant inflammation which leads to fever in nearly all infections. If the major viral tropism is for the CNS, virus reaching this site by crossing the blood–brain barrier or along neural pathways can cause meningeal inflammation (aseptic meningitis) or neuronal dysfunction (encephalitis). The CNS pathology consists of meningeal and perivascular mononuclear cell infiltrates; degeneration of neurons with neuronophagia; and occasionally, destruction of the supporting structure of neurons.

Liver often the target, with necrosis of hepatocytes

In some infections, especially yellow fever, the liver is the primary target organ. Pathologic findings include hyaline necrosis of hepatocytes, which produces cytoplasmic eosinophilic masses called **Councilman bodies.** Degenerative changes in the renal tubules and myocardium may also be seen, as may microscopic hemorrhages throughout the brain. Hemorrhage is a major feature of yellow fever, largely because of the lack of liver-produced clotting factors as a result of liver necrosis.

Dengue hemorrhagic fevers involve perivascular and endothelial injury

May progress to shock

Hemorrhagic fevers other than those related to primary hepatic destruction have a somewhat different pathogenesis which has been studied most extensively in dengue infections. In uncomplicated dengue fever, which is associated with a rash and influenza-like symptoms, there are changes in the small dermal blood vessels. These alterations include endothelial cell swelling and perivascular edema with mononuclear cell infiltration. More severe infection, as in dengue hemorrhagic fever, often complicated by shock, is characterized by perivascular edema and widespread effusions into serous cavities such as the pleura and hemorrhages from the upper respiratory and intestinal tracts. The spleen and lymph nodes show hyperplasia of lymphoid and plasma cell elements, and there is focal necrosis in the liver. The pathophysiology seems related to increased vascular permeability and disseminated intravascular coagulation, which is further complicated by liver and bone marrow dysfunction (eg, decreased platelet production, decreased production of liver-dependent clotting factors). The major vascular abnormalities may be provoked by circulating virus–antibody complexes (immune complexes) that mediate activation of complement and subsequent release of vasoactive amines. The precise reason for this phenomenon is not clear; it may be related to intrinsic virulence of the virus strains involved and to host susceptibility factors.

Lymphoid hyperplasia seen

Virus–antibody complexes may trigger complement activation

Two hypotheses are based on the existence of four distinct but antigenically related serotypes of dengue virus, any of which can generate group-specific cross-reacting antibodies that are not necessarily protective against other serotypes. One possibility is that preexisting group-specific antibody at a critical concentration serves as "enhancing" rather than neutralizing antibody. In the presence of enhancing antibody, virus–antibody complexes are more efficiently adsorbed to and engulfed by monocytes and macrophages. Subsequent replication leads to extensive spread throughout the host. Alternatively, or in concert with this, activation of previously sensitized T cells by viral antigen present on the surfaces of macrophages may result in release of cytokines, which mediate the development of shock and hemorrhage.

Cross reacting antibodies may enhance infection

## IMMUNITY

The usual humoral responses (hemagglutination inhibition, complement fixation, neutralization, precipitation) in relation to onset of illness are illustrated in Figure 40–1. The rise in antibody titer generally correlates with recovery from infection. Neutralizing antibodies, which are the most serotype specific, generally persist many years after infection. The presence of IgM-specific antibodies indicates that primary infection likely occurred within the previous 2 months. Cellular and humoral immunity to reinfection are serotype specific and appear to be permanent.

Neutralizing antibodies protective and last for years

Immunity is serotype specific

## ARBOVIRUS DISEASE: SPECIFIC ARBOVIRUSES

### Western Equine Encephalitis

The agent that causes western equine encephalitis is prevalent in the central valley of California, eastern Washington (Yakima valley), Colorado, and Texas. It has also been responsible for outbreaks in midwestern states (Minnesota, Wisconsin, Illinois, Missouri, and Kansas) and as far east as New Jersey. Horses and humans represent blind-end hosts;

Human and equine illness

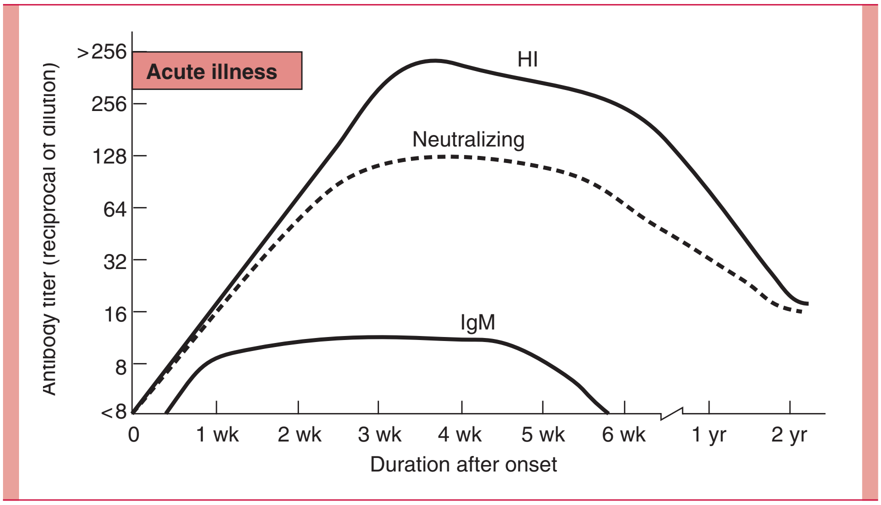



FIGURE 40–1
Typical patterns of antibody response after arbovirus infection. HI, hemagglutination inhibition antibodies; IgM, immunoglobulin M antibodies, begin to appear about 3 days after onset and disappear after about 6 weeks.

both are susceptible to infection and illness, commonly manifested as encephalitis. Although human infection in endemic areas is commonplace, overall only 1 of 1000 infections causes clinical symptoms. However, in young infants, 1 of every 25 infections may produce severe illness. The attack rates are therefore far higher in young infants than in other groups. The disease spectrum may range from mild, nonspecific febrile illness to aseptic meningitis or severe, overwhelming encephalitis. Mortality is estimated at 5% for cases of encephalitis. It is a very serious disease in infants less than 1 year of age; as many as 60% of survivors have permanent neurologic impairment.

Encephalitis is more likely in young infants

## Eastern Equine Encephalitis

The eastern equine encephalitis virus is largely confined to the Atlantic Seaboard states from New England down the coasts of Central America and South America. The mosquito vector (principally *Culiseta melanura*) generally restricts its feeding to horses and birds, although occasional outbreaks among humans have occurred. The virus can cause severe encephalitis in horses and also in wild birds. The mortality among humans is estimated at 50% for individuals of all ages, and the incidence of severe sequelae among survivors is high.

New England to South America

Vector feeds on horses and birds

## St. Louis Encephalitis

The St. Louis encephalitis virus is a major cause of arbovirus encephalitis in the United States. Its geographic distribution and major mosquito vector (*Culex tarsalis*) are similar to those of western equine encephalitis, but has been much more prevalent in eastern states and in Texas, Mississippi, and Florida. It infects but causes no disease in horses. The disease spectrum in humans is similar to that of western equine encephalitis, but the major morbidity and mortality, as well as the highest attack rates, are among adults more than 40 years of age. Infants and young children are relatively spared.

Distribution and disease is similar to western equine encephalitis

More disease seen in adults

## West Nile Virus

During the summer of 1999 in the northeastern United States, human West Nile virus infections appeared for the first time in the Western Hemisphere. A subsequent outbreak occurred again in 2000. Together, these outbreaks resulted in 78 hospitalized patients and 9 deaths, mostly among the elderly. More widespread activity was observed in 2001 (66 human cases); then in 2002, a dramatic increase in virus spread was seen across the United States, with activity in 46 states and four Canadian provinces. There were at least 3600 human cases reported in the United States, with 212 deaths. Prior to 1999, outbreaks of human infections were primarily confined to eastern Africa, the Middle East, eastern Europe, west Asia and Australia.

First appeared in United States in 1999

The virus is antigenically related to St. Louis encephalitis and Japanese encephalitis. Transmission is from infected mosquitoes to birds, humans and horses, and clinical illness leading to death can result from infections in any of these hosts. Transmission among humans via blood transfusions, breast milk, or organ transplants is also possible. Crows are particularly affected; virus has been detected in dead crows found as far south as Florida, and more recently in the midwestern United States. Clinical illness in the United States has often included muscle weakness and flaccid paralysis, suggesting an axonal polyneuropathy in addition to encephalitis.

Dead crows often herald spread of virus in nature

Muscle weakness and flaccid paralysis can occur

## California Virus

Although California virus was first isolated in that state, its major distribution in the United States has been in the Midwest; outbreaks due to the LaCrosse subtype are particularly prevalent in Wisconsin, Ohio, Minnesota, Indiana and West Virginia. In Wisconsin and Minnesota, California virus is considered the most important cause of encephalitis. However, studies elsewhere in North America and throughout the world, indicate that California virus or closely related agents are present nearly everywhere. The primary mosquito vector (*Aedes triseriatus*) is commonly encountered in suburban or rural environments. Unlike western equine, eastern equine, and St. Louis encephalitis viruses, the highest attack rates are seen in those aged 5 to 18 years. Infection is often characterized by abrupt onset of encephalitis, frequently with seizures.

Virus and vector common in suburban and rural areas

Highest attack rate in those aged 5 to 18 years

## Yellow Fever

Widespread in tropical areas

Vector persists in United States

Geographically, yellow fever is distributed throughout the Caribbean and Central America, the Amazon valley in South America, and a broad central zone in Africa from the Atlantic Coast to the Sudan and Ethiopia. It continues to be a potential threat to the southeastern United States because of an urban vector (*Aedes aegypti*) in that area. The clinical disease is characterized by abrupt onset of fever, chills, headache, and hemorrhage. It may progress to severe vomiting (sometimes with gastric hemorrhage), bradycardia, jaundice, and shock. If the patient recovers from the acute episode, there are no long-term sequelae.

## Dengue

Vector same as yellow fever

There are four related serotypes of dengue, any of which may exist concurrently in a given endemic area. These agents are widespread throughout the world, particularly in the Middle East, Africa, the Far East, and the Caribbean Islands, and they have invaded the United States in the past. The vector (*Aedes aegypti*) is the same as the domestic vector of yellow fever. The known transmission cycle is human–mosquito–human, although a sylvatic cycle involving monkeys may also exist.

Severe pain in back, muscles, and joints

The characteristic clinical illness usually results in fever, an erythematous rash, and severe pain in the back, head, muscles, and joints. Especially in the Far East (Philippines, Thailand, and India), the disease has periodically assumed a severe form characterized by shock, pleural effusion, and hemorrhage often followed by death.

## Japanese B Encephalitis

Transmission is similar to St. Louis and western equine encephalitis

The flavivirus species that causes Japanese B encephalitis is prevalent on the eastern coast of Asia, on its offshore islands (Japan, Taiwan, and Indonesia), and in India. Its transmission cycle resembles that of the St. Louis encephalitis and western equine encephalitis viruses. A high proportion of human infections are subclinical, especially in children; when encephalitis does develop it is severe and often fatal.

## Powassan Virus

Tick-borne but uncertain human importance

Powassan virus is the only known tick-borne *Flavivirus* species of North America. First isolated in Ontario from a fatal human case of encephalitis, it has been found in infected ticks in Ontario, British Columbia, and Colorado. Its significance to humans is not yet established; only a few patients with encephalitis proved to be caused by this agent have been described. However, serologic evidence suggests that the virus is prevalent in many areas of North America.

## Colorado Tick Fever

Tick-borne throughout western United States

Most infections asymptomatic

The tick-borne *Orbivirus* species that causes Colorado tick fever has been found throughout the western United States, including Washington, Oregon, Colorado, and Idaho, and also Long Island. It is frequently found in *Dermacentor andersoni,* which are also vectors for *Rickettsia rickettsii.* The typical illness, which occurs 3 to 6 days after the tick bite, is characterized by a sudden onset with headache, muscle pains, fever, and occasionally encephalitis. Leukopenia is a consistent feature of infection. It is estimated that no more than one clinical illness occurs for every 100 infections with this agent.

# ARBOVIRUS DISEASE: CLINICAL ASPECTS

## DIAGNOSIS

The arboviruses may be isolated in various culture systems including intracerebral inoculation of newborn mice, which often results in encephalitis and death. The viruses may be found in the blood (viremia) from a few days before onset of symptoms through the first 1 to 2 days of illness; attempts at isolation from the blood are generally useful only when viremia is prolonged, as in dengue, Colorado tick fever, and some of the hemorrhagic fevers. Virus is not present in the stool and is rarely found in the throat; viral recovery from cerebrospinal fluid (CSF) is also unusual. Virus can be detected in CSF or affected tissue by reverse transcriptase polymerase chain reaction, and sometimes by culture during the acute phase of illness. Specific diagnosis is usually accomplished by serologic techniques using acute and convalescent sera. Various tests have been used including hemagglutination inhibition, complement fixation, virus neutralization methods, and enzyme immunoassay. Early rapid presumptive diagnosis can sometimes be made by the detection of IgM-specific antibodies that often appear within a few days of onset (except in Colorado tick fever, where they may be delayed by 1 to 2 weeks), and persist 1 to 2 months.

Blood is best source but must be early in disease

Multiple serologic methods used

## TREATMENT AND PREVENTION

There is generally no specific treatment for arboviral infections other than supportive care; ribavirin has been used on occasion, but controlled studies have not been reported to support or refute its effectiveness. Prevention is primarily avoidance of contact with potentially infected arthropods, a task that can be extremely difficult even with the use of adequate screening and insect repellents. In some settings, vector control can be accomplished by elimination of arthropod breeding sites (stagnant pools and the like) and sometimes by attempts to eradicate the arthropods with careful use of insecticides. Such measures have been highly effective in the control of urban yellow fever, in which elimination of urban breeding sites and other measures to eradicate the principal mosquito vector species (*Aedes aegypti*) have been used. Viruses maintained in complex sylvatic cycles are infinitely more difficult to control without risking major environmental disruption and inestimable expense.

Treatment is only supportive

Protection from bites and vector control are primary prevention

Vaccines are available for immunization of horses against western, eastern, and Venezuelan equine encephalitis virus infections, and the latter has also been used for some laboratory personnel who work with the virus. The only other arbovirus vaccine in general use for humans is a live attenuated yellow fever virus vaccine (17-D strain), which is used to protect rural populations exposed to the sylvatic cycle and international travelers to endemic areas. In fact, many countries in tropical Africa, Asia, and South America require proof of yellow fever vaccination before allowing travelers to enter.

Yellow fever vaccine is available

# OTHER RNA VIRUSES OF ZOONOTIC ORIGIN

## ARENAVIRUSES

A common feature of the arenaviruses is their zoonotic reservoir, particularly small rodents, in which they may be sustained for long periods. Primary infection (horizontal transmission) in mature rodents often results in disease and death, whereas intrauterine or perinatal infection (vertical transmission) usually leads to chronic lifelong viremia with persistent shedding of virus into the feces, urine, and respiratory secretions. Although chronically infected rodents are somewhat tolerant to the virus (ie, infection is persistent without causing illness), they produce antibodies, and evidence of deleterious effects can be found in older hosts, usually in the form of immune complex glomerulonephritis. The viruses are perpetuated by vertical transmission from infected mothers to their offspring. When environmental contact becomes close, spread from the rodent reservoir to humans (and, in some instances, subhuman primates) can occur via aerosols; through exposure to infective urine, feces, or tissues; or directly by rodent bites. This is in contrast to the arthropod spread of arboviruses.

Sustained in small rodent reservoirs

Vertical transmission in rodents

Spread to humans by aerosols and close contact

## Arenaviruses Associated with Hemorrhagic Fevers

Person-to-person spread occurs by contact with body fluids

The agents of arenavirus hemorrhagic fevers are transmitted from infected rodents to humans in the manner described above, although person-to-person spread by contact with secretions and body fluids also occurs readily. The viruses in this group include the South American hemorrhagic fever agents (Junin virus, the cause of Argentinean hemorrhagic fever, and Machupo virus, the cause of Bolivian hemorrhagic fever) and Lassa virus, the cause of **Lassa fever** in West Africa.

All cause fever, shock, and hemorrhage

Hepatitis and myocarditis also occur with Lassa fever

High mortality and risk of further transmission

These viruses have pathogenic and pathologic features similar to those described for the arboviruses that cause hemorrhagic fevers; however, the mechanism involved in the coagulation abnormalities is not understood. All are characterized by fever, usually accompanied by hemorrhagic manifestations, shock, neurologic disturbances, and bradycardia. Lassa fever also frequently causes hepatitis, myocarditis, exudative pharyngitis, and acute deafness. The last deficit may persist after recovery. Mortality is estimated to be 10 to 50% for Lassa fever and 5 to 30% for the others. All are considered highly dangerous in terms of infectivity. Importation of cases to nonendemic areas has occurred, with significant risk of spread to medical and laboratory personnel.

Suggested by clinical findings and travel history

Diagnosis only in reference centers

Viremia may be prolonged

The diagnosis is suggested primarily by the recent travel history of the patient and the clinical syndromes. Although virus isolation and serologic diagnosis may be performed, these procedures should not be attempted in a hospital diagnostic laboratory. Any patient suspected of having such an infection should be immediately isolated and public health authorities notified. Because of the high risk of spread of infection from body fluids and excreta, even routine laboratory studies are best deferred until the diagnosis and proper disposition of specimens can be resolved. Viremia can persist 1 month, and virus shedding in the urine may continue more than 2 months after the onset of illness. Treatment is primarily supportive; however, intravenous ribavirin, if begun within 6 days of illness onset, has been shown to be helpful in Lassa fever.

## Lymphocytic Choriomeningitis Virus

Transplacental infection in humans

Infection with lymphocytic choriomeningitis virus is particularly common in hamsters and mice. In the United States, most human illnesses have been traced to contact with rodent breeding colonies in research or pet supply centers and to pet hamsters in the home. The illness usually consists of fever, headache, and myalgia although meningitis or meningoencephalitis also occurs occasionally. Such CNS infections may persist as long as 3 months. There is also evidence that transplacental infection can occur in humans, resulting in fetal death, hydrocephalus, or chorioretinitis. No person-to-person transmission of infection has been documented.

Mice and hamsters in pet stores

Meningitis may persist for months

The diagnosis is suggested by a history of rodent contact. The virus may be isolated in the early stages of disease by cell culture or intracerebral inoculation of blood or CSF into weanling mice or young guinea pigs. Serologic testing of acute and convalescent sera is usually performed by indirect immunofluorescence.

# FILOVIRUSES: MARBURG AND EBOLA VIRUSES

Initial cases transmitted from monkeys

The association of the Marburg virus with serious disease did not become apparent until 1967, when 26 cases of hemorrhagic fever occurred among persons in Germany and Yugoslavia who were handling a group of African monkeys imported from central Uganda. The agent was later identified as Marburg virus and was apparently transmitted by the infected monkeys. In 1975 the virus was associated with a similar disease in three travelers in South Africa, and in 1980 in Kenya.

Viruses differ antigenically

In 1976, severe outbreaks of hemorrhagic fever occurred in northern Zaire and southern Sudan, with case fatality rates from 50 to 90%. The illnesses were similar to those described for Marburg virus but were later shown to be caused by an antigenically different agent known as Ebola virus, named after a river in Zaire. More recently, another filovirus serologically related to Ebola virus was isolated from monkeys during an epizootic of simian hemorrhagic fever at a US quarantine facility. The reservoir was determined to be monkeys imported from the Philippines.

The reasons why these viruses can cause such fulminant, lethal hemorrhagic disease with shock in humans are not entirely clear. There is evidence that Marburg virus replicates in vascular endothelial cells, with subsequent necrosis. Other researchers have also shown that Ebola virus may exert its effects via a glycoprotein, synthesized in either a secreted or transmembrane form. The secreted glycoprotein interacts with neutrophils to inhibit early activation of the inflammatory response, while the transmembrane glycoprotein binds to endothelial cells. Ebola virus produces disease in humans and subhuman primates; onset is within 4 to 6 days of inoculation. The reservoir, although uncertain, is thought to be in small mammals, perhaps rodents. Serosurveys of humans residing in the areas where outbreaks have occurred suggest that human infections may be relatively common; as much as 7% of the survey group had antibodies, indicating past infection. In symptomatic infections, the mortality for both Marburg and Ebola viruses is extremely high (30 to 80%).

Reservoir may be small mammals

Mortality high in symptomatic infection

As with the arenavirus-associated hemorrhagic fevers, the diagnosis of infection by these agents is suggested by a similar syndrome and recent travel history. Person-to-person transmission similar to that described for Lassa fever occurs in Ebola virus infections and may be possible with Marburg virus. Diagnosis can be confirmed in a reference center by isolation of virus, as well as by serologic methods employing indirect immunofluorescence or EIA. However, as with the arenavirus-associated hemorrhagic fevers, utmost care in isolation precautions and prompt notification of public health authorities are mandatory for suspected cases before any diagnostic attempts are made. There is no specific therapy for the infections.

Diagnosis and precautions similar to arenavirus hemorrhagic fevers

## HANTAVIRUSES

### Hantavirus Hemorrhagic Fever

Korean hemorrhagic fever (KHF) is endemic to Korea and surrounding areas in the Far East. It is an important cause of hemorrhagic fever, often complicated by varying degrees of acute renal failure. In the 1950s, thousands of military personnel developed the disease during the Korean War. The first reported isolation of KHF was in 1978, when the antigen was detected in the lung tissues of wild rodents (*Apodemus* species) by indirect immunofluorescence using convalescent sera from affected patients. No illness was apparent in the rodents, suggesting a reservoir mechanism and mode of transmission similar to those described for the arenaviruses. Additional work indicated that the agent is a member of the family Bunyaviridae, and the generic designation of *Hantavirus* was given.

Causes of hemorrhagic fever during Korean War

Detected in lung of wild rodents

Evidence has accumulated indicating that other agents with close antigenic similarities to KHF virus are responsible for hemorrhagic–renal syndromes occurring throughout northern Eurasia, including Russia, Eastern Europe, Finland, and Scandinavia. These syndromes have been given a variety of names, including nephropathia epidemica. Methods similar to those used to detect KHF have detected nephropathia epidemica antigen in the lungs of small rodents (bank voles) in Finland.

Other viruses similar to KHF throughout northern Eurasia

### Other *Hantavirus* Infections

It has been known for some time that rodents in the United States may be infected with a hantavirus, but no associated human disease was recognized. In early 1993, an outbreak of fulminant respiratory disease with high mortality (50 to 75%) occurred in the southwestern United States. This syndrome (hantavirus pulmonary syndrome, or HPS) has been related to at least three hantaviruses, of which Sin Nombre virus is the most common. Infections are associated with an increased population of infected mice in and around human habitations. Of the more than 30 documented HPS illnesses reported in 1993, 23 patients resided in rural areas of a region bordered by the states of Arizona, New Mexico, and Colorado; however, cases have also been reported from at least 19 other states. The virus is believed to be transmitted to humans most often by inhalation of infected rodent excreta, by the conjunctival route, or by direct contact with skin breaks. Human-to-human spread has not been encountered. Public health measures to inform inhabitants of routes of spread and to reduce the rodent population appear to have controlled the outbreak.

Hantavirus among rodents in United States

Southwestern US outbreak related to deer mice

Humans infected by inhalation of aerosolized excreta

No human-to-human transmission

Ribavirin may be useful

Treatment has involved aggressive respiratory support. Intravenous ribavirin appears to have been of benefit in Asian hantavirus infections; however, there are no data as yet regarding its efficacy against the US strains.

## VESICULAR STOMATITIS VIRUS

A rhabdovirus, vesicular stomatitis virus, that causes outbreaks of disease in cattle, pigs, and horses can be transmitted between animals by arthropods. Human infection is acquired by contact with infected animals but is unusual; it consists of a self-limited febrile illness and occasional herpes-like eruptions over the lips and mucosa.

## ADDITIONAL READING

Chen Y, Maguirre T, Hileman RE, et al. Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nature Med* 1997;3:866–871. This report illustrates how determining the nature of viral receptors facilitates understanding of viral pathogenesis and possible strategies for treatment.

Jahrling PB, Peters CJ. Lymphocytic choriomeningitis virus. A neglected pathogen of man. *Arch Pathol Lab Med* 1992;116:486–488. The history, unique biology, and clinical features of this virus are well summarized.

Johnson RT. Acute encephalitis. *Clinical Infect Dis* 1996;23:219–226. For the clinically curious, this article discusses the features of arbovirus encephalitides comparing them with other causes of brain inflammation.

Kautner I, Robinson MJ, Kuhnle U. Dengue virus infection: Epidemiology, pathogenesis, clinical presentation, diagnosis, and prevention. *J Pediatr* 1997;131:516–524. An excellent clinical review.

Khan AS, Khabbaz RF, Armstrong LR, et al. Hantavirus pulmonary syndrome: The first 100 U.S. cases. *J Infect Dis* 1996;173:1297–1303. A thorough review of the syndrome.

Martin AA, Gubler DJ. West Nile encephalitis: An emerging disease in the United States. *Clinical Infect Dis* 2001;33:1713–1719. A review of events that transpired and what might be expected in the future.

McCormick JB, King IJ, Webb PA, et al. Lassa fever. Effective therapy with ribavirin. *N Engl J Med* 1986;314:20–26. This article demonstrates approaches and difficulties encountered in evaluating a new drug for a serious disease.

McJunkin JE, Khan R, de los Reyes EC, et al. Treatment of severe LaCrosse encephalitis with intravenous ribavirin following diagnosis by brain biopsy. *Pediatrics* 1997;99:261–267. This intriguing case report illustrates how the laboratory can be used effectively in diagnosing and managing a serious disease.

Yang Z, Delgado R, Xu L, et al. Distinct cellular interactions of secreted and transmembrane Ebola virus glycoproteins. *Science* 1998;279:1034–1037. Insight is provided for how this virus may disarm host cell defenses and cause severe damage to the vascular endothelium.

CHAPTER 41

# Rabies

W. LAWRENCE DREW

Rabies is an acute fatal viral illness of the central nervous system (CNS). The word rabies is derived from the Latin verb to rage, which suggests the appearance of the rabid patient. It can affect all mammals and is transmitted between them by infected secretions, most often by bite. It was first recognized more than 3000 years ago and has been the most feared of infectious diseases. It is said that Aristotle recognized that rabies could be spread by a rabid dog.

## VIROLOGY

The rabies virus is a bullet-shaped, enveloped, single-stranded RNA virus of the rhabdovirus group (Fig 41–1). Other pathogens in this group include the vesicular stomatitis virus (see Chapter 40). Rabies virus is large, with dimensions of about 180 by 70 nm. Knob-like glycoprotein excrescences, which elicit neutralizing and hemagglutination-inhibiting antibodies, cover the surface of the virion. In the past, a single antigenically homogeneous virus was believed responsible for all rabies; however, differences in cell culture growth characteristics of isolates from different animal sources, some differences in virulence for experimental animals, and antigenic differences in surface glycoproteins have indicated strain heterogeneity among rabies virus isolates. These studies may help to explain some of the biological differences as well as the occasional case of "vaccine failure."

RNA virus is bullet-shaped

Strains from different sources are antigenically heterogeneous

## RABIES

CLINICAL CAPSULE

Rabies involves the development of severe neurologic symptoms and signs in a patient who was previously bitten by an animal. The neurologic abnormalities are very characteristic, with a relentlessly progressive excess of motor activity, agitation, hallucinations, and salivation. The patient appears to be foaming at the mouth and has severe throat contractions if swallowing is attempted. The neurologic abnormalities are explained by spread of the virus from the bite wound into the CNS and then centrifugally to the autonomic nervous system.

### EPIDEMIOLOGY

Rabies exists in two epizootic forms, urban and sylvatic. The urban form is associated with unimmunized dogs or cats, and the sylvatic form occurs in wild skunks, foxes,

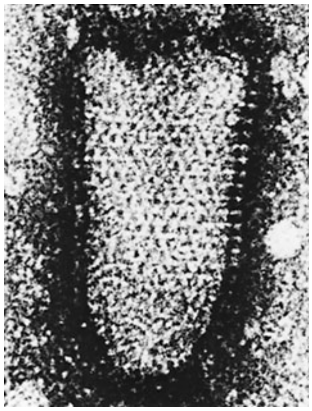


FIGURE 41–1
Rabies virus. (*Reprinted with permission from Dr. K. Hummular, from Hummuler K, Koprowski M, Wiktor TJ.* J Virol *1967;1:152–170.*)

wolves, raccoons, and bats but not rodents or rabbits. Introduction of an infected animal into a different geographic area can lead to infection of many new members of that species. For example, raccoon hunters apparently are to blame for the sudden appearance of raccoon strain rabies in West Virginia and Virginia in 1977. Prior to that time, the nearest cases of raccoon rabies were found several hundred miles away in South Carolina. The hunters are believed to have imported infected raccoons from another state. Since 1977, raccoon rabies has spread from West Virginia and Virginia to 12 northeastern states.

Human infection, or the much more common infection of cattle, is incidental, is blind-ended, and does not contribute to maintenance or transmission of the disease. In the United States, more than 75% of reported cases of rabies in animals occur among wildlife. Human exposures may be from wild animals or from unimmunized dogs or cats. In recent years, there has been a decrease of US cases to less than two per year, and bat exposure has been the source in almost all cases despite a resurgence of rabies in skunks and raccoons. An occasional case has resulted from aerosol exposure (eg, bat caves and no bite). Domestic animal bites are very important sources of rabies in developing countries because of lack of enforcement of animal immunization. Infection in domestic animals usually represents a spillover from infection in wildlife reservoirs. Human infection tends to occur where animal rabies is common and where there is a large population of unimmunized domestic animals. Worldwide, the occurrence of human rabies is estimated to be about 15,000 cases per year, with the highest attack rates in Southeast Asia, the Philippines, and the Indian subcontinent.

Risks to humans are from bites by infected carnivores, omnivores, and bats

Aerosol spread from exposure in bat caves

## PATHOGENESIS

The essential first event in human or animal rabies infection is the inoculation of virus through the epidermis, usually as a result of an animal bite. Inhalation of heavily contaminated material, such as bat droppings, can also cause infection. Rabies virus first replicates in striated muscle tissue at the site of inoculation. Immunization at this time is presumed to prevent migration of the virus into neural tissues. In the absence of immunity, the virus then enters the peripheral nervous system at the neuromuscular junctions and spreads to the CNS, where it replicates exclusively within the gray matter. It then passes centrifugally along autonomic nerves to reach other tissues, including the salivary glands, adrenal medulla, kidneys, and lungs. Passage into the salivary glands in animals facilitates further transmission of the disease by infected saliva. The neuropathology of rabies resembles that of other viral diseases of the CNS, with infiltration of lymphocytes and plasma cells into CNS tissue and nerve cell destruction. The pathognomonic lesion is the Negri body (Fig 41-2), an eosinophilic cytoplasmic inclusion distributed throughout the brain, particularly in the hippocampus, cerebral cortex, cerebellum, and dorsal spinal ganglia.

Replicates initially in muscle and then enters peripheral nervous system

Spreads to CNS gray matter

Negri bodies found in neurons

The incubation period ranges from 10 days to a year, depending on the amount of virus introduced, the amount of tissue involved, the host immune mechanisms, the innervation of the site, and the distance the virus must travel from the site of inoculation

Incubation period can be prolonged for months

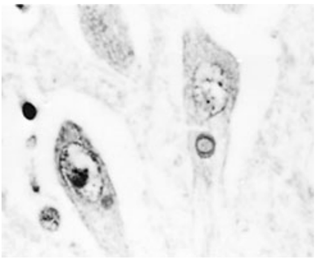


FIGURE 41–2
Negri body in cytoplasm of neuron.
(*Courtesy of Dr. Daniel P. Perl.*)

to the CNS. Thus, the incubation period is generally shorter with face wounds than with leg wounds. Immunization early in the incubation period frequently aborts the infection.

## RABIES: CLINICAL ASPECTS

### MANIFESTATIONS

Rabies in humans usually results from a bite by a rabid animal or contamination of a wound by its saliva. It presents as an acute, fulminant, fatal encephalitis; human survivors have been reported only occasionally. After an average incubation period of 20 to 90 days the disease begins as a nonspecific illness marked by fever, headache, malaise, nausea, and vomiting. Abnormal sensations at or around the site of viral inoculation occur frequently and probably reflect local nerve involvement. The onset of encephalitis is marked by periods of excess motor activity and agitation. Hallucinations, combativeness, muscle spasms, signs of meningeal irritation, seizures, and focal paralysis occur. Periods of mental dysfunction are interspersed with completely lucid periods; however, as the disease progresses, the patient lapses into coma. Autonomic nervous system involvement often results in increased salivation. Brainstem and cranial nerve dysfunction is characteristic, with double vision, facial palsies, and difficulty in swallowing. The combination of excess salivation and difficulty in swallowing produces the fearful picture of "foaming at the mouth." Hydrophobia, the painful, violent involuntary contractions of the diaphragm and accessory respiratory, pharyngeal, and laryngeal muscles initiated by swallowing liquids, is seen in about 50% of cases. Involvement of the respiratory center produces respiratory paralysis, the major cause of death. Occasionally rabies may appear as an ascending paralysis resembling Guillain–Barré syndrome. The median survival after onset of symptoms is 4 days, with a maximum of 20 days unless artificial supportive measures are instituted. Recovery is rare and has only been seen in partially immunized individuals.

Encephalitis common, sometimes with ascending paralysis

Almost uniformly fatal

### DIAGNOSIS

The CSF of a rabies patient shows minimal to no abnormalities with some patients exhibiting a lymphocytic pleocytosis (5 to 30 cells/$mm^3$). The test of choice in a live patient is detection of rabies antigen by immunofluorescent stain of a nape of the neck biopsy. PCR of CSF or saliva may supplant the neck biopsy. Laboratory diagnosis of rabies in animals or deceased patients is accomplished by demonstration of virus in brain tissue. Viral antigen can be demonstrated rapidly by immunofluorescence procedures. Intracerebral inoculation of infected brain tissue or secretions into suckling mice results in death in 3 to 10 days. Histologic examination of their brain tissue shows Negri bodies in 80% of cases; electron microscopy may demonstrate both Negri bodies and rhabdovirus particles. Specific antibodies to rabies virus can be detected in serum but generally only late in the disease.

Virus or antigen detected in brain tissue

### TREATMENT

Prevention is the mainstay of controlling human rabies. Intensive supportive care has resulted in two or three long-term survivals; despite the best modern medical care, however, No specific treatment is available

the mortality still exceeds 90%. In addition, because of the infrequency of the disease, many cases die without definitive diagnosis. Human hyperimmune antirabies globulin, interferon, and vaccine do not alter the disease once symptoms have developed.

## PREVENTION

Vaccine-induced antibody inhibits viral spread

In the late 1800s Pasteur, noting the long incubation period of rabies, suggested that a vaccine to induce an immune response before the development of disease might be useful in prevention. He apparently successfully vaccinated Joseph Meister, a boy severely bitten and exposed to rabies, with multiple injections of a crude vaccine made from dried spinal cord of rabies-infected rabbits. This treatment emerged as one of the best known and most noteworthy accomplishments in the annals of medicine. It is now believed that vaccination induces antibody that is either neutralizing or inhibits cell to cell spread of virus. Natural infection does not lead to an early immune response and limitation of viral migration, because the virus is replicating in muscle or neural tissue and lymphocytes do not access these sites. Cytotoxic T lymphocytes are also induced by vaccine and appear to be directed against an antigen of the virus.

High-risk individuals include veterinarians, spelunkers, laboratory workers

Currently, the prevention of rabies is divided into preexposure and postexposure prophylaxis. Preexposure prophylaxis is recommended for individuals at high risk of contact with rabies virus, such as veterinarians, spelunkers, laboratory workers, and animal handlers. The vaccine currently used in the United States for preexposure prophylaxis employs an attenuated rabies virus grown in human diploid cell culture and inactivated with $\beta$-propiolactone. Preexposure prophylaxis consists of two subcutaneous injections of vaccine given 1 month apart, followed by a booster dose several months later.

Careful history and studies of biting animal are important in decision-making

Postexposure prophylaxis requires careful evaluation and judgment. Every year more than one million Americans are bitten by animals, and in each instance a decision must be made whether to initiate postexposure rabies prophylaxis. The physician must consider (1) whether the individual came into physical contact with saliva or another substance likely to contain rabies virus; (2) whether there was significant wounding or abrasion; (3) whether rabies is known or suspected in the animal species and area associated with the exposure; (4) whether the bite was provoked or unprovoked (i.e., the circumstances surrounding the exposure); and (5) whether the animal is available for laboratory examination. Any wild animal or ill, unvaccinated, or stray domestic animal involved in a possible rabies exposure, such as an unprovoked bite, should be captured and killed. The head should be sent immediately to an appropriate laboratory, usually at the state health department, for search for rabies antigen by immunofluorescence. If examination of the brain by this technique is negative for rabies virus, it can be assumed that the saliva contains no virus and that the exposed person requires no treatment. If the test is positive, the patient should be given postexposure prophylaxis. It should be noted that rodents and rabbits are not important vectors of rabies virus.

Rabies immune globulin plus vaccine necessary in postexposure management

Postexposure prophylaxis is based on immediate, thorough washing of the wound with soap and water; passive immunization with hyperimmune globulin, of which at least half the dose should be instilled around the wound site; and active immunization with antirabies vaccine. With human diploid vaccine, five doses given on days 1, 3, 7, 14, and 28 are recommended. Physicians should always seek the advice of the local health department when the question of rabies prophylaxis arises.

## ADDITIONAL READING

Advisory Committee on Immunization Practices (ACIP). Rabies prevention–United States 1999. *Morb Mortal Wkly Rep* 1999;48(RR-1):1-22. This report provides specific guidelines for rabies prophylaxis.

Fishbein DB, Robinson LE. Rabies. *N Engl J Med* 1993;329:1632–1638. A review of the epidemiology of rabies, including changes in animal reservoirs in differing geographical locations.

CHAPTER 42

# Retroviruses, Human Immunodeficiency Virus, and Acquired Immunodeficiency Syndrome

JAMES J. CHAMPOUX AND W. LAWRENCE DREW

Retroviruses are enveloped, single-stranded plus-sense RNA viruses. They encode an enzyme called **reverse transcriptase** that converts the RNA genome into a double-stranded DNA copy that subsequently becomes integrated into the host cell DNA. Representatives of two major groups are considered in this chapter: the **oncoviruses** (*onco-*, "related to a tumor") and the **lentiviruses** (*lenti-*, "slow"). Like most enveloped viruses, all retroviruses are highly susceptible to factors that affect surface tension and are thus not transmissible through air, dust, or fomites under normal conditions, but instead intimate contact with the infecting source is required.

Enveloped RNA viruses that encode reverse transcriptase

Members of the oncovirus subgroup of retroviruses have long been associated with a variety of cancers in animals, including leukemias, lymphomas, and sarcomas, but until recent years had not been found to infect humans. The first human retrovirus, human T-cell leukemia virus type I (HTLV-1), was discovered in the late 1970s. It was shown to cause adult T-cell leukemia, a rare malignancy found only in Japan, Africa, and the Caribbean, although serologic evidence shows that the virus also occurs in the United States and has raised the possibility of an association with some chronic neurologic conditions. A relative of HTLV-1, HTLV-2, has been associated with a few rare cases of T-cell malignancies, including hairy cell leukemia, but its precise role in these diseases remains unclear.

Oncoviruses cause tumors in many animals

HTLV-1 and -2 associated with human leukemias

The most important disease resulting from a human retrovirus infection, **acquired immunodeficiency syndrome (AIDS),** is caused by either of two lentiviruses termed human immunodeficiency viruses types 1 and 2 (**HIV-1** and **HIV-2**). A devastating disease, for which there is no present cure, AIDS has spurred unprecedented research efforts to determine the nature and pathogenic mechanisms of the viruses in the hope of finding effective drugs and vaccines. Most of our present knowledge of HIV is derived from studies on HIV-1, which is the major cause of AIDS worldwide.

HIV-1 and -2 are lentiviruses that cause AIDS

Oncoviruses do not kill the cell they infect, but instead they continue to produce new virus indefinitely. This property, combined with the fact that they can transduce growth-promoting genes called **oncogenes** into a recipient cell, accounts in part for their

Oncoviruses usually not cytolytic; they transduce or activate oncogenes ability to cause malignancies (see Chapter 7 and below). With lentivirus infections, the cell–virus relationship is quite different. Lentiviruses can apparently persist for long periods of time in a latent state without causing much cell killing, only to become highly cytolytic when the infected cells are subjected to certain stimuli. The prototype lentivirus is visna virus, which causes a slow degenerative neurologic disease in sheep. Like visna, HIV-1 can remain latent in the infected cell without serious effects, but when induced to replicate, high levels of virus are produced and the cell dies. Although HIV-1 can infect a variety of human cell types, its most drastic effects appear to result from destruction of the CD4+ subclass of T lymphocytes, which play a central role in the capacity of the host to mount effective and protective immunologic responses to a wide range of infections.

Lentiviruses can become cytopathic after latent period

HIV attacks and destroys CD4+ T lymphocytes

# RETROVIRUSES

## VIROLOGY

### STRUCTURE

All retroviruses are remarkably similar in their basic composition. The structure of HIV-1 is depicted in Fig 42–1. The virion is about 100 nm in diameter, and because it contains two copies of the RNA genome, it is diploid. The RNA genome is coated with the nucleocapsid protein (NC), and the RNA–protein complexes are enclosed in a capsid (CA) composed of multiple subunits. Like all enveloped viruses, the membrane is acquired during budding from the host cell, but the surface (SU, also called gp120) and transmembrane (TM, also called gp41) glycoproteins found in the envelope are virally encoded. Between the capsid and the envelope is the matrix (MA) protein. In addition to the structural proteins shown in Fig 42–1, the virion core contains three virus-specific proteins that are essential for viral replication: reverse transcriptase (RT), protease (PR), and integrase (IN). The relationships between the viral genes found in all retroviruses (*gag, pol,* and *env*) and the proteins they encode are presented in Table 42–1. Some retroviruses, including HIV-1, encode additional regulatory and accessory proteins as will be described below.

Virion contains two single-stranded RNA molecules

Envelope acquired during budding contains two viral glycoproteins

### LIFE CYCLE

Figure 42–2 depicts the life cycle of a typical retrovirus and serves to illustrate the many unique aspects of retroviral replication that could be potential targets of therapeutic intervention.

### Viral Entry

The virions adsorb to cellular membrane receptors and enter the cell by direct fusion with the plasma membrane. For HIV-1, the virion attachment protein is the SU glycoprotein

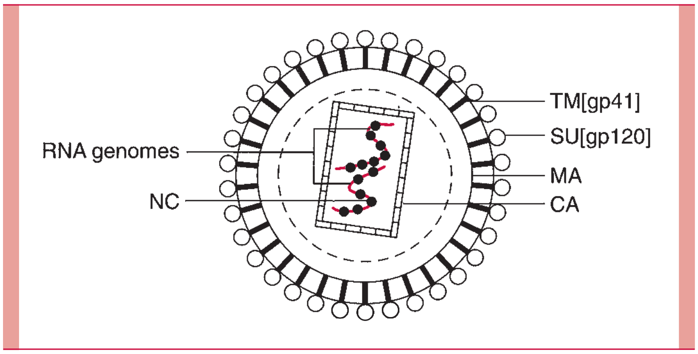



FIGURE 42–1
Structure of HIV particle. The two RNA molecules enclosed within the capsid (CA) are coated with the nucleocapsid protein (NC). The matrix protein (MA) lies just inside the membrane envelope. The envelope contains two membrane glycoproteins, gp41 and gp120, also called transmembrane protein (TM) and surface protein (SU), respectively.

TABLE 42–1

**Major Retroviral Genes and Proteins**

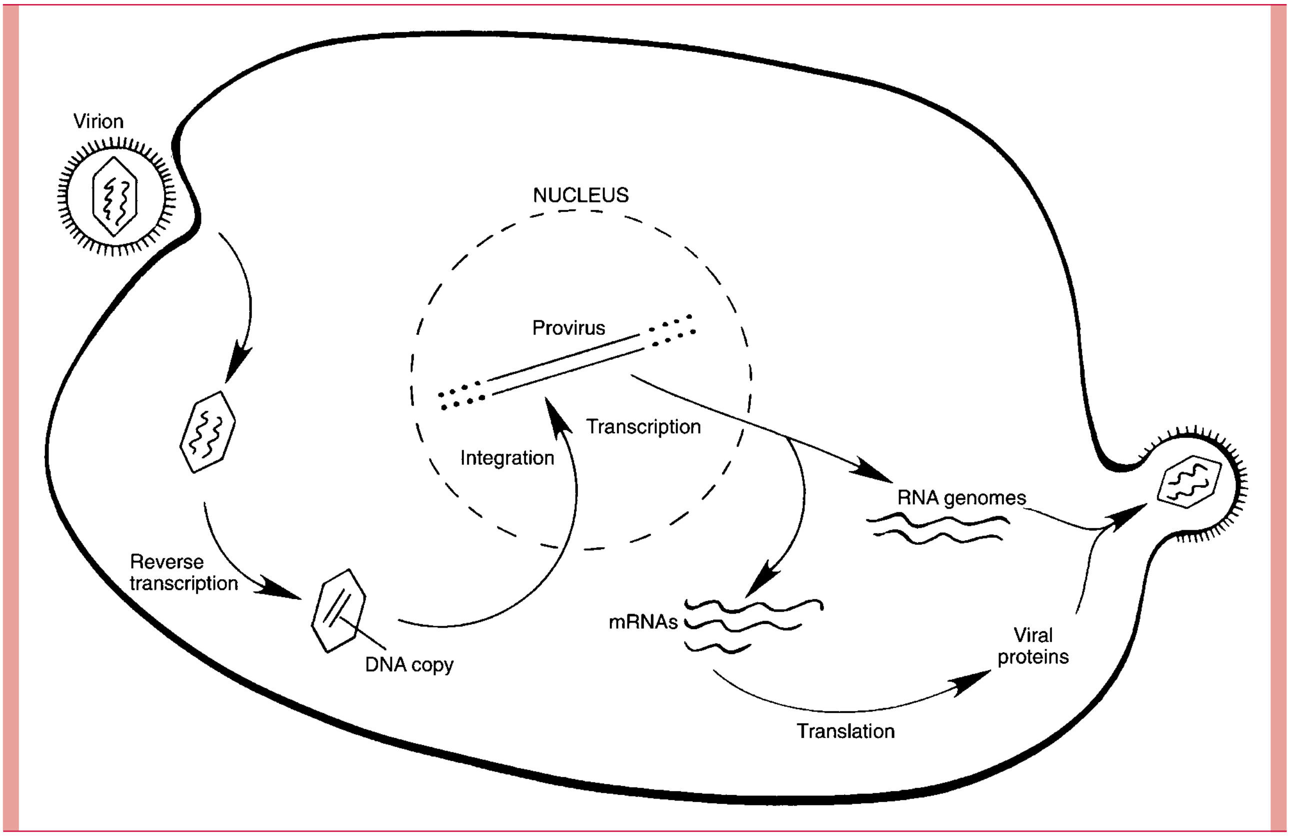
| Gene[a] | Protein Products | Function |
|---|---|---|
| *gag* | Matrix (MA) | Structural |
| | Capsid (CA) | Structural |
| | Nucleocapsid (NC) | Structural |
| | Protease[b] (PR) | Protein processing |
| *pol* | Protease[b] (PR) | Protein processing |
| | Reverse transcriptase (RT) | DNA synthesis |
| | Integrase (IN) | Integration |
| *env* | Surface glycoprotein (SU) | Adsorption |
| | Transmembrane protein (TM) | Fusion of envelope with plasma membrane |

[a] Each gene encodes a polyprotein that is subsequently processed by proteolysis to yield the individual proteins.
[b] The protease is encoded in either the *gag* gene or the *pol* gene, depending on the virus.

gp120, and the cellular receptor is the CD4 molecule with one of the chemokine receptors, CXCR4 or CCR5 acting as coreceptors. These receptors occur primarily in the plasma membrane of CD4+ T lymphocytes, cells of the monocyte–macrophage series, and some other target cells. Early in the infection of an individual, the viruses are often macrophage-tropic because they preferentially use the CCR5 coreceptor. The emergence of syncytia-forming variants that use the CXCR4 coreceptor and are T-cell tropic appears

HIV-1 surface (SU) glycoprotein gp120 attaches to CD4 cell and chemokine coreceptors

FIGURE 42–2
Retroviral life cycle.



Transmembrane (TM) gp41 protein mediates fusion of viral and cell membranes

to correlate with rapid advancement to AIDS. The HIV-1 transmembrane TM protein gp41 is responsible for fusion of the viral and cell membranes, leading to entry of the virion core complex into the cytoplasm of the cell.

Can infect cells without CD4 molecule

Fusion provides direct cell-to-cell transmission

HIV-1 can also infect cells such as fibroblasts and certain brain cells that lack the CD4 surface molecule, apparently because the chemokine receptors in combination with the fusion-inducing activity of the TM protein is sufficient in these cases to promote entry. Fusion activity may also play an important role in amplification of the effects of the virus infection, particularly during the later stages of the infection, because infected cells expressing viral glycoproteins in their membranes readily fuse with uninfected CD4+ T lymphocytes to form large syncytia. This process appears to provide a means for cell-to-cell transmission of the virus that bypasses the usual extracellular phase and may contribute to the overall depletion of CD4+ lymphocytes in an infected individual.

## Viral RNA Replication

Reverse transcriptase copies RNA to double-stranded DNA

DNA integrates into host chromosome and replicates with the cell as a provirus

Among the RNA viruses, retroviral replication is unique. Soon after entry of the viral core into the cytoplasm of the infected cell, the RNA is copied into double-stranded DNA by reverse transcriptase, the virion-associated DNA polymerase. The overall process is referred to as **reverse transcription** and results in a linear DNA molecule that enters the nucleus and integrates more-or-less at random into a host cell chromosome. Once the viral genetic information has been converted to DNA and integrated, it essentially becomes part of the cellular genome. The viral genes, called the **provirus,** are therefore replicated and faithfully inherited as long as the infected cell continues to divide.

Provirus includes its own promoter and signals that control transcription by host RNA polymerase

Genomic RNA and spliced mRNAs are both produced: the latter encode envelope glycoproteins and regulatory proteins

HIV-1 can control extent of genomic or spliced mRNA production

Special sequences contained within the RNA are duplicated during the reverse transcription process so that the integrated provirus contains identical long terminal repeats (LTRs) at its ends. The LTR sequences contain the appropriate promoter, enhancer, and other signals required for transcription of the viral genes by the host RNA polymerase II. Transcription produces a full-length RNA genome and one or more spliced mRNAs. For the oncoviruses, the predominant spliced mRNA is translated to produce the envelope glycoproteins, but in HIV-1, a series of spliced mRNAs are produced that encode, in addition to the envelope proteins, a series of viral regulatory and accessory proteins. Unlike most retroviruses, HIV-1 and the other lentiviruses apparently exert considerable control over whether the primary transcripts are allocated to full-length RNA or are spliced to produce mRNAs (see below). With the exception of these regulatory and accessory proteins, all retroviral proteins are initially translated as polyproteins that are subsequently processed by proteolysis into the individual protein molecules. The enzyme responsible for most of these protein cleavages is the virus-specific protease (PR) that is encoded in either the *gag* gene or the *pol* gene, depending on the virus (see Table 42–1).

RNase H activity degrades original RNA genome

Integrase-catalyzed integration is random in host DNA

Integrated DNA is transcribed by host RNA polymerase

A simplified view of retroviral RNA replication is presented in Figure 42–3. In addition to DNA polymerase activity, the reverse transcriptase possesses an RNase H activity that is responsible for degrading the RNA portion of the DNA–RNA hybrid (+RNA/−DNA) produced in the first phase of reverse transcription. The immediate product of reverse transcription is a linear double-stranded DNA molecule that is flanked by the LTR sequences. The viral integrase (IN) catalyzes the integration of the linear DNA into host DNA. The integration process is highly specific with respect to the viral DNA, and two base pairs are generally lost from each end of the DNA. The choice of a target site for integration into the cellular DNA appears, however, to be nearly random. A short sequence of base pairs in the target DNA (four to six, depending on the virus) is duplicated during the integration process, and these repeat sequences immediately flank the integrated provirus. The replication process is completed by transcription of the proviral DNA by the host RNA polymerase II.

It should be noted that the scheme represented in Figure 42–3 also describes the replication cycle for hepatitis B virus (see Chapter 37). Instead of packaging the RNA form of the genome as occurs with retroviruses, hepatitis B virus packages the double-stranded DNA that is the immediate product of reverse transcription.

Of all the known retroviruses, HIV-1 possesses the most error-prone reverse transcriptase. The consequence of this high error rate is that each time the viral RNA is reverse

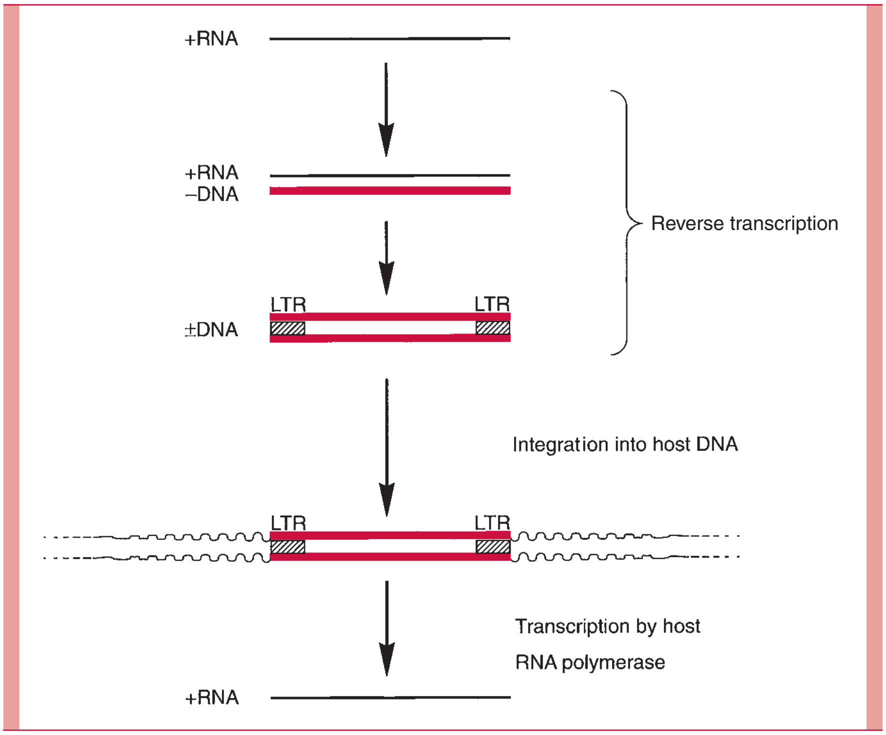



FIGURE 42–3
Retroviral RNA replication. LTR, long terminal repeat.

transcribed, one to two new mutations are introduced into the resulting DNA. Because the process of transcription of the integrated proviral DNA to produce new viral genomes is also error prone, mutant genomes accumulate rapidly over the course of an infection. The end result is a quasispecies that accounts for the many nucleotide differences observed between different isolates (even from the same infected individual) and for the variability of the SU envelope protein gp120. It may explain, in part, the failure of the immune system to control the infection and also the increases in viral virulence that appear to occur during the course of the infection.

HIV reverse transcriptase is error prone

Isolates from the same patient can differ in multiple properties

## RETROVIRAL GENES

The organization of the genome of different types of retroviruses is shown in Figure 42–4 (see also Table 42–1). The order of the genes for a typical retrovirus is *gag–pol–env.* The *gag* (group-specific antigen) gene encodes the structural proteins of the virus and, in some cases, the protease. The *pol* (polymerase) gene encodes the reverse transcriptase, the integrase, and sometimes the protease. The *env* (envelope) gene encodes the two membrane glycoproteins found in the viral envelope. Not surprisingly, the SU protein (gp120 in HIV-1) is responsible for the host range of the virus and its antigenicity.

Genome is organized into *gag, pol,* and *env* genes

The genomes of acute transforming oncoviruses have a variety of structures, but one feature is common to nearly all of them: some viral genes are replaced by genes derived from their hosts that render them oncogenic (see below). In every case, the signals required for reverse transcription and for transcription of the provirus, which are located near the ends of the RNA, are retained in the infecting virus. In the example shown in Figure 42–4, the *pol* gene and parts of both the viral *gag* and *env* genes are deleted, but other configurations are possible. Such oncoviruses are defective and replicate only in the presence of a helper virus that can supply the missing functions.

Some retroviruses carry host genes rendering them oncogenic

Defective transforming oncogenic viruses require helper virus

A comparison of the genetic makeup of HIV-1 with that of a typical retrovirus (see Fig 42–4) reveals a larger number of genes and a much more complex organization. HIV-1 contains, in addition to the *gag, pol,* and *env* genes, an array of other genes (*tat, rev, nef, vif, vpr,* and *vpu*). Expression of these genes requires mRNA splicing, and all apparently encode proteins that serve regulatory or accessory roles during the infection (see below). HTLV-1 encodes the regulatory proteins, Tax and Rex, which are analogous to the HIV-1

HIV-1 has multiple regulatory genes

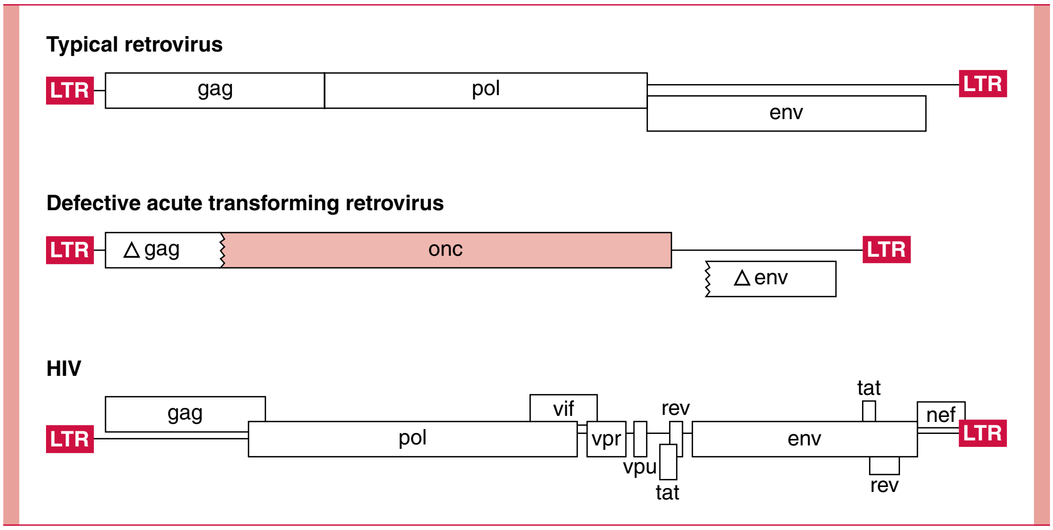



FIGURE 42–4
Maps of the integrated forms of various retroviral genomes are drawn with the genes and long terminal repeats (LTRs) shown as boxes. The vertical displacements of the boxes above and below the lines depict the different reading frames of the coding segments.

Tat and Rev proteins. The names of the genes that have been best characterized and the proteins and functions they determine are listed in Table 42–2.

## TRANSFORMATION BY RETROVIRUSES

Oncogenic retroviruses appear to transform cells to an oncogenic state by three distinct mechanisms (see Chapter 7).

Noncytocidal viruses carrying cellular oncogenes can produce persistent transformation

First, the defective acute transforming viruses (see Fig 42–4) have acquired a cellular gene (thereafter called an **oncogene**) that when expressed in the infected cell results in loss of normal growth control. On infection, the transduced oncogene is expressed from the viral LTR promoter, resulting in a rapid and acute onset of malignant disease. Persistent transformation by oncogene transduction is possible only for those retroviruses that are not cytocidal. More than 30 different oncogenes have been identified in a variety of animal retroviruses, but no human retroviruses are known that transform by this mechanism.

Integration adjacent to cellular protooncogenes can activate them

The second mechanism is called **insertional mutagenesis.** Integration of a retrovirus in the vicinity of particular cellular genes can cause inappropriate expression of the gene, resulting in uncontrolled cell growth. These cellular genes are called

TABLE 42–2

### HIV-1 Regulatory and Accessory Proteins

| Gene | Protein | Function |
|---|---|---|
| *tat* | Tat | Transcriptional activator |
| *rev* | Rev | Promotes transport of unspliced mRNAs |
| *nef* | Nef | Downregulation of cellular CD4 and MHC I proteins |
| *vpu* | Vpu | Facilitate virus assembly and release |
| *vpr* | Vpr | Facilitates nuclear entry in nondividing cells |
| *vif* | Vif | Increases viral infectivity in certain cell types |

*Abbreviations*: MHC, major histocompatibility complex.

**protooncogenes,** and insertional activation by the virus is apparently due to the close proximity of the integrated viral promoter or enhancer to the gene. Cancers that are caused by this mechanism have very long latent periods, because integration is random and only rarely occurs near a cellular protooncogene.

The causative agent of adult T-cell leukemia, HTLV-1, exemplifies the third mechanism. In this case, the integrated provirus in the leukemic cells from any one patient is found at a unique location on a particular chromosome. Thus, the tumors are probably monoclonal. The cancer is not the result of insertional activation, however, because the chromosomal location of the provirus is never the same in any two patients. Instead, transformation results from the continual expression of the viral *tax* gene (the HTLV-1 homolog of the HIV-1 *tat* gene; see Table 42–2). Apparently, the Tax protein not only can transactivate viral transcription in the same manner as Tat (see below), but Tax can also **transactivate** the expression of one or more cellular genes (possibly protooncogenes), resulting in malignant transformation.

HTLV-1 transforms by production of Tax, which activates cellular transforming genes

## ROLES OF HIV-1 REGULATORY AND ACCESSORY PROTEINS

A unique feature of HIV-1 and other members of the lentivirus subfamily is the ability to produce a complex array of regulatory and accessory proteins that appear to be responsible for staging the infection, increasing the efficiency and yield of the infection, and in some cases contributing to viral latency. These proteins also appear to interact with cellular factors to modulate the infection differently in different host cells. The roles of the two HIV-1 regulatory genes, *tat* and *rev,* and the four accessory proteins, *nef, vpu*, *vpr,* and *vif,* are discussed below and summarized in Table 42–2. Although the four accessory proteins are dispensable in many cell culture systems, they appear to be important for the maximum pathogenic potential of the virus in infected individuals.

The products of the *tat* and *rev* regulatory genes are the Tat and Rev proteins, respectively. Both of these proteins have the effect of staging the infection, so that in the absence of abundant transcription of the proviral genome, only limited gene expression is possible. When the infected T-lymphocyte is stimulated, for example by antigen presentation, Tat and Rev play a positive role in promoting viral gene expression. In the absence of high levels of Tat, the host RNA polymerase initiates properly at the LTR promoter, but transcription is usually prematurely terminated leading to the production of short, dead-end transcripts. Tat is a transcriptional activator that acts at a sequence near the beginning of the viral mRNA, called TAR, to recruit cellular proteins to the transcribing RNA polymerase, resulting in a modification to the polymerase that prevents premature termination and allows complete transcription of the proviral genome.

Tat is transcriptional activator that promotes synthesis of full-length viral transcripts

The Rev protein acts at the level of mRNA splicing. Normally, unspliced cellular transcripts are retained in the nucleus and only fully spliced mRNAs are transported to the cytoplasm for translation. The only viral proteins that are made from fully spliced mRNAs are Tat, Rev, and Nef, and consequently only these proteins are found early after infection, when there is no mechanism to prevent complete splicing of pre-mRNAs. To express the Vif, Vpr, and Vpu proteins, and the Env polyprotein, which are all made from singly spliced transcripts, as well as the Gag and Pol polyproteins, which are translated from the unspliced genomic RNA, it is necessary to transport incompletely spliced RNAs to the cytoplasm. Transport of partially spliced transcripts is accomplished by Rev binding to a site on the viral RNA within the *env* gene called the Rev-responsive element (RRE). The RNA-bound Rev then interacts with normal cellular machinery responsible for protein export from the nucleus to mediate the movement of the RNA through the nuclear pore. By promoting translation of the virion structural proteins and some of the accessory proteins, Rev turns up late gene expression that leads directly to a high rate of virus production.

Rev promotes export of unspliced and partially spliced transcripts to cytoplasm

The Nef accessory protein enhances virus production and virion infectivity and also appears to interfere with immune recognition of infected cells. Nef causes the internalization and degradation of the CD4 protein, which likely contributes to virus release by preventing the formation of complexes between the cellular receptor and newly synthesized

Nef downregulates CD4 to promote virus release and also downregulates MHC I to interfere with immune recognition

virions. Nef also causes the downregulation of cell surface major histocompatibility complex (MHC) I molecules, which may prevent recognition of infected cells by cytotoxic T lymphocytes. In addition, virions produced in the absence of the Nef protein are at least partially blocked at some step prior to integration. The combination of these and perhaps other effects allows the Nef protein to play an essential pathogenic role in an infected individual.

Vpu targets CD4 destruction and virion release

The Vpu protein appears to play two separate roles during the late stages of infection. In the absence of Vpu, the Env protein forms complexes with CD4 in the endoplasmic reticulum and fails to reach the plasma membrane of the cell. One of the roles of Vpu is to target the destruction of CD4 in the endoplasmic reticulum to allow for incorporation of Env into newly synthesized virions. The second role of Vpu is to promote the release of virions from the infected cell by an unknown mechanism.

Vpr promotes transport of subviral particles into nucleus of nondividing cells

The Vpr protein is involved in promoting import of subviral particles into the nucleus after reverse transcription. Thus, the protein has little or no effect in proliferating T cells where nuclear access is ensured with each mitosis. However, successful infection of nondividing cells such as macrophages requires Vpr to allow the newly synthesized viral DNA to reach the nucleus and be integrated into the cellular DNA.

Vpu and Vif increase efficiency of infection and yield of virus

Vif (virion infectivity factor) increases the infectivity of HIV-1 in primary T cells and certain "nonpermissive" cells in culture. In the absence of Vif, the virus fails to complete reverse transcription in these cell types. "Permissive" cell lines infected by mutants defective in the *vif* gene produce normal yields of infectious virus. One possible explanation for this observation is that "permissive" cells contain a factor that can substitute for the missing Vif protein. Thus, one role of Vif may be to extend the host range of HIV-1 to cell types that would otherwise not be infected.

Acativation of CD4+ T lymphocytes increases virus production

Superimposed on this complex regulatory network is the fact that the viral promoter contains elements that are sensitive to specific cellular transcription factors. This observation may help explain why virus production in CD4+ T lymphocytes is greatly increased when the cells are activated. Clearly the outcome of an HIV-1 infection is determined by a complex interplay between a very large number of different factors.

# ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

CLINICAL CAPSULE

The primary infection in AIDS ranges from asymptomatic to an infectious mononucleosis-like illness with up to a few weeks of fever, malaise, arthralgias, and rash. A long (years) asymptomatic period follows, after which the disease, AIDS, emerges. The progressive findings directly due to the virus are wasting, diarrhea, neurologic degeneration, and malignancies. The effect of the virus on the immune system causes an extensive array of viral, bacterial, fungal, and parasitic opportunistic infections whose findings are the same or worse than those seen in patients without AIDS.

## EPIDEMIOLOGY

First recognized in male homosexuals, hemophiliacs, and drug abusers

The AIDS syndrome was first recognized in the United States in 1981, when it became apparent that an unusual number of rare skin cancers (Kaposi's sarcoma) and opportunistic infections were occurring among male homosexuals. These patients were found to have a marked reduction in CD4+ T lymphocytes and were subject to a wide range of opportunistic infections normally controlled by an intact immune system. The disease was found to progress relentlessly to a fatal outcome and was first identified in male homosexuals, hemophiliacs who were receiving blood-derived coagulation factors, and injection drug users.

Retrospective serologic studies with material saved from patients in various studies indicate that the disease was already occurring in Africa in the 1950s and in the United States in the 1970s. In 1985, HIV-2 was found to be endemic in parts of West Africa and to cause AIDS. To date, this virus has been relatively restricted geographically, although HIV-2 infections have occurred in the western hemisphere.

HIV-2 is endemic to West Africa

## Transmission

The HIV virus is transmitted between humans in three ways: sexually, perinatally, and by exposure to contaminated blood or body fluids. The virus has been demonstrated in particularly high titers in semen and cervical secretions, and the majority of cases result from sexual contact. Infection is facilitated by breaks in epithelial surfaces, which provide direct access to the underlying tissues or bloodstream. The relative fragility of the rectal mucosa, together with large numbers of sexual contacts, are probable contributing factors to the predominance of the disease among promiscuous male homosexuals. Transmission appears to be more efficient from men to women, but the reverse is clearly documented. The probability of HIV transmission per unprotected sexual act is estimated at 0.0003 to 0.0015. The risk of perinatal transmission from an infected mother to her child has been estimated to range from 15 to 40%.

Transmission is sexual and by exposure to infective fluids

Perinatal transmission can readily occur

Growth of the virus in cell culture and identification of its antigens allowed development of effective test procedures for detecting HIV infection. These almost eliminated the risk of transmission by blood transfusion; testing of donors and the use of recombinant or specially treated coagulation factors have now virtually eliminated these sources of infection. Until serologic tests for the infection became available, in 1985, more than 10,000 cases of AIDS were probably acquired in the United States through blood transfusion, and about 80% of hemophiliacs treated with coagulation factors derived from pooled blood sources became infected. Transmission of infection by blood is now largely associated with sharing of needles and syringes by injecting drug users, and this has been an increasing source of the disease. In some areas of the world, the seroprevalence of HIV positivity among injecting drug users has been as high as 70%. It became apparent that heterosexual transmission could occur and that the infection could be transmitted from mother to infant either by intrauterine spread or during the birth process. It was also found that the disease had its greatest prevalence in parts of Africa, where the spread was predominantly heterosexual.

Testing of blood supply reduced risk

Intravenous drug abusers are at extremely high risk

Transmission of infection to health care workers after accidental sticks with potentially contaminated needles is very rare (considerably less than 1% of occurrences), presumably because the amount of infectious virus in the blood of infected cases is small and larger volumes or repeated exposures are needed for a significant chance of infection. Nevertheless, cases have occurred from both clinical and laboratory exposure, and extreme care in handling needles, sharps, and so on, is necessary. Transmission does not occur through day-to-day nonsexual contact with infected individuals or through insect vectors, because of the fragility of the virus and the need for direct mucosal or blood contact. It is of interest that the virus has been detected in saliva, tears, urine, and breast milk. With the possible exception of breast milk, these sources have not been shown to be infectious.

Accidental needlesticks among health care workers mandate extreme care in prevention

Shed in breast milk, where it may infect breastfeeding infants

## Occurrence

As of December 2001, there have been 816,000 cases of AIDS in the United States, with 468,000 deaths. The highest prevalence rates of HIV infections have been in homosexual and bisexual males, intravenous drug users, prostitutes, and sexual partners of HIV-infected persons. In some areas of the United States, 40 to 60% of homosexual males attending sexually transmitted disease clinics were found to be infected. The epidemiology of HIV infection is changing in the United States as the pandemic evolves and as the modes of transmission become more generally understood. The numbers and proportions of heterosexually transmitted, drug abuse–related, and neonatal cases are increasing, particularly among the poor and disadvantaged racial minorities. Antibody rates in prostitutes may be as high as 40%, depending partly on the degree of associated intravenous drug abuse. Prevalence rates in the

Prevalence rates have shifted over time, with increasing cases among women and economically disadvantaged minority groups heterosexual population, in general, are currently less than 1% but have been increasing. In 1985 in the United States, only 7% of AIDS cases were in women; by 2000 the percentage had risen to 25%. Approximately 2000 newborns per year are infected by HIV perinatally, but this number may be decreasing as more pregnant women receive antiretroviral therapy. The current distribution of AIDS cases is men who have sex with men (MSM) (40%), intravenous drug users (30%), heterosexual (25%) persons, and others (5%). Black patients now account for 50% of cases, exceeding the percentages in non-Hispanic white men.

Men and women nearly equally inffected in Africa and Asia

In contrast to the situation in the United States and Western Europe, heterosexual transmission is the primary route of transmission in Africa and Asia, where there is an approximately equal distribution of infection and disease between the sexes. This may be due to a high frequency in these areas of ulcerative genital lesions caused by other sexually transmitted diseases. These lesions facilitate passage of virus into the tissues of others during intercourse. In central and eastern Europe, where there is an emerging epidemic, the most common risk factor is intravenous drug use.

Increasingly widespread in Africa, South America, parts of Asia

AIDS has been reported in more than 150 countries. The disease continues to spread rapidly in Africa and South America. In sub-Saharan Africa alone, 25 million people are infected, and there are 4 million new cases per year. Until recently, the Far East had few cases, but now there is epidemic spread, especially in South and South East Asia (India, South China, Burma, Thailand, Cambodia, Viet Nam, and Malaysia). In China, there are more than 600,000 patients with AIDS, and the rate of new cases is increasing by more than 30% per year. HIV-2 infection is found primarily in West Africa and is spread by heterosexual transmission. Infection by this virus has, however, been reported in Europe in homosexual men, injection drug users, transfusion recipients, and hemophiliac men. For example, in Russia, there were 40,000 new cases of AIDS in 2000. In some countries in Africa, 25% of the population and up to 60% of women are HIV antibody–positive.

## PATHOGENESIS

The pathogenesis of HIV-1 infection is very complex, but the following factors are likely to be important in the disease-causing process.

### Infection

Major targets are CD4-bearing cells, but other cell types can also be infected

The initial target of HIV-1 is CD4 molecules, particularly on the surface of CD4+ helper T lymphocytes, monocytes, and macrophages. The virus can also infect other human cells expressing CD4, and a wide range of CD4 negative cells, including renal and gastrointestinal epithelium and brain astrocytes. The mechanism for infection of non–CD4-bearing cells is unknown but may involve other receptors or fusion with cells already infected with HIV. The virus replicates in macrophages, and these cells could serve as a reservoir for continued expansion of the infection to other cell types by cell-to-cell fusion, which allows the virus to spread without being exposed to neutralizing antibody. Infected macrophages may participate in breakdown of the blood–brain barrier, allowing enhanced exposure of the central nervous system (CNS). Although CNS and intestinal disturbances are a prominent part of fully developed AIDS, it is not clear whether they are a direct result of infection of these cells or mediated by cytokines from infected macrophages and T lymphocytes.

Rapid turnover of CD4+ cells during infection

Kinetic studies of changes in viral load with antiviral therapy demonstrated the half-life of HIV in plasma is 5 to 6 hours. In other words, more than 50% of the viral load measured on any given day has been produced in the past 24 hours. Because 99% of the viral load is produced by cells that were infected within the past 48 to 72 hours, cell turnover must be equally rapid. Indeed, when similar kinetic studies are performed on changes in CD4 cell counts, it is estimated that up to 1 billion CD4 cells are produced per day in response to the infection and that the half-life of these cells is only 1.6 days.

### Latency

The long asymptomatic period following HIV infection (clinical latency) occurs despite active virus replication in the host. Several factors can terminate the long latent period of

HIV-1. Mutations occur during viral replication that appear to enhance induction of virulent forms of the virus, with increased cytopathic capacity and altered cell tropisms. Thus, the mutated forms of HIV-1 isolated from later stages of disease infect a broader range of cell types and grow more rapidly than those isolated in the asymptomatic period. Initially, it was believed that little or no viral replication occurred during this latent period, but studies of lymph nodes of individuals with early asymptomatic disease have shown intense immunologic reactions within the lymphoid tissue at early stages of disease. This implies that the immune system is capable of controlling the virus to some degree early in the course of disease, an ability that is later lost as the disease progresses over time.

Some immune control of virus during the latent period, but this is later lost

Recent studies of HIV infection have shown that the level of free virus in the plasma increases in direct relation to the stage of disease. Individuals with early-stage disease have less than 10 infectious virions/mL of plasma, whereas those in late-stage disease have between 100 and 1000 infectious virions/mL of plasma. These studies imply either that viral replication was increasing during later stages of disease due to more virulent mutations and/or the immune system had lost its ability to clear free virus as the disease progresses.

Level of plasma viremia directly correlates with disease progression

## Immune Deficiency

The primary immune defect in AIDS results from the reduction in the numbers and effectiveness of CD4+ helper-inducer T lymphocytes, both in absolute numbers and relative to CD8+ suppressor T lymphocytes. This is due to direct killing of CD4+ T lymphocytes by the virus but may also involve other mechanisms as well. These include secondary killing of uninfected (bystander) cells during cell fusion, autoimmune processes that lead to the elimination of CD4+ T lymphocytes by opsonophagocytosis, and antibody-dependent cell-mediated cytotoxicity (ADCC) directed at gp120 expressed on the CD4+ cell surface. There are also functional defects in CD4+ T lymphocytes affecting lymphokine production and leading to inhibition of some macrophage functions.

Immune deficiency related to reduction in numbers and normal functions of CD4+ T lymphocytes

Effects on CD4+ T lymphocytes thus lead to a generalized failure of cell-mediated immune responses, but there is also an effect on antibody production due to polyclonal activation of B cells, possibly associated with other viral infections of these cells. This overwhelms the capacity of infected individuals to respond to specific antigens. The end result of these processes is a disturbance of immune balance that can give rise to malignancies as well as the susceptibility of AIDS patients to a range of opportunistic viral, fungal, and bacterial infections.

Inffected individuals are susceptible to other infections and malignancies

# ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS): CLINICAL ASPECTS

## MANIFESTATIONS

In 1993, the Centers for Disease Control and Prevention (CDC) definition of AIDS stated that all patients who are HIV antibody–positive and have CD4+ T-lymphocyte counts below $200/mm^3$ or less than 14% of total T lymphocytes have the disease. The initial infection with HIV is usually asymptomatic, although in some cases a mononucleosis-like illness develops 2 to 4 weeks after infection and lasts about 2 to 6 weeks. This illness may exhibit any or all of the following: fever, malaise, lymphadenopathy, hepatosplenomegaly, arthralgias, and rash. Sometimes a mild aseptic meningitis is also present. Whether or not these early manifestations of infection occur, the virus persists and integrates into the genome of some host cells, and the individual is thus infected for life.

Infection is lifelong

The initial infection is followed by an asymptomatic period that, in most cases, continues for years before the disease becomes clinically apparent. During this time virus can be isolated from blood, semen, and the cervix. Approximately 50% of infected individuals develop significant disease within 10 years of infection, and the number continues to increase thereafter. It is expected that nearly all HIV-infected individuals eventually

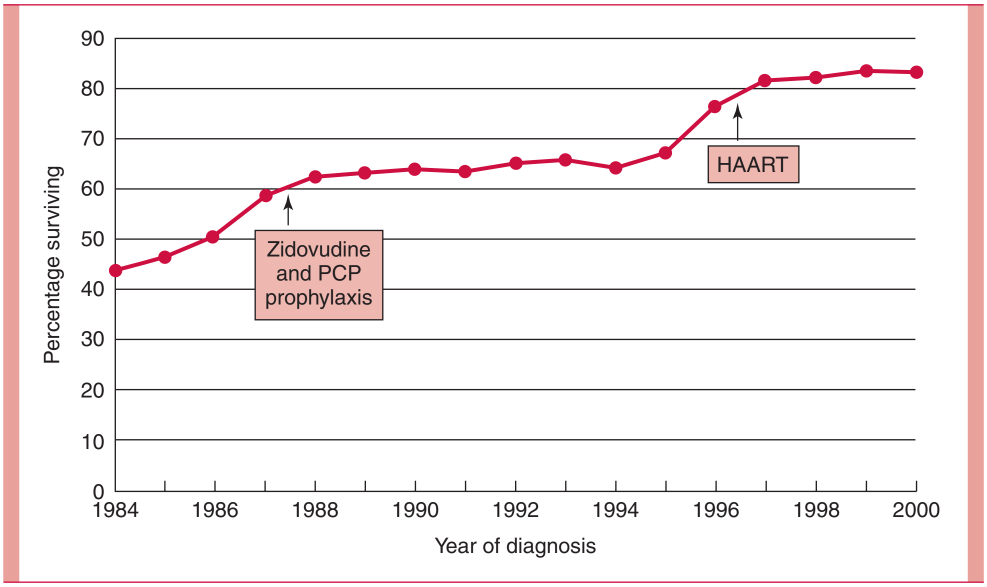



FIGURE 42–5

Proportion of AIDS patients surviving at least 1 year after diagnosis of their first AIDS-defining opportunistic illness, by year of diagnosis of opportunistic illness, 1984–2000, United States. HAART, highly active antiretroviral therapy; PCP *Pneumocystis carinii* pneumonia. (*From Centers for Disease Control and Prevention, National Center for HIV, STD, and TB Prevention, Divisions of HIV/AIDS Prevention. Surveillance Supplemental Report 2002, Vol 8, No 1.*)

Progression to AIDS is highly variable among individuals

develop some clinical aspects of this infection, although long-term (>1 years) nonprogressors are well documented. Approximately 5% of infected, untreated patients show no decrease in CD4 counts over a period of more than 10 years, but ultimately many of these individuals begin to progress. However, since the late 1990s, the increases in early diagnosis, combined with more aggressive, highly active antiretroviral therapy (HAART) in the United States, have shown promise in delaying progression of infection to death (Figs 42–5, 42–6).

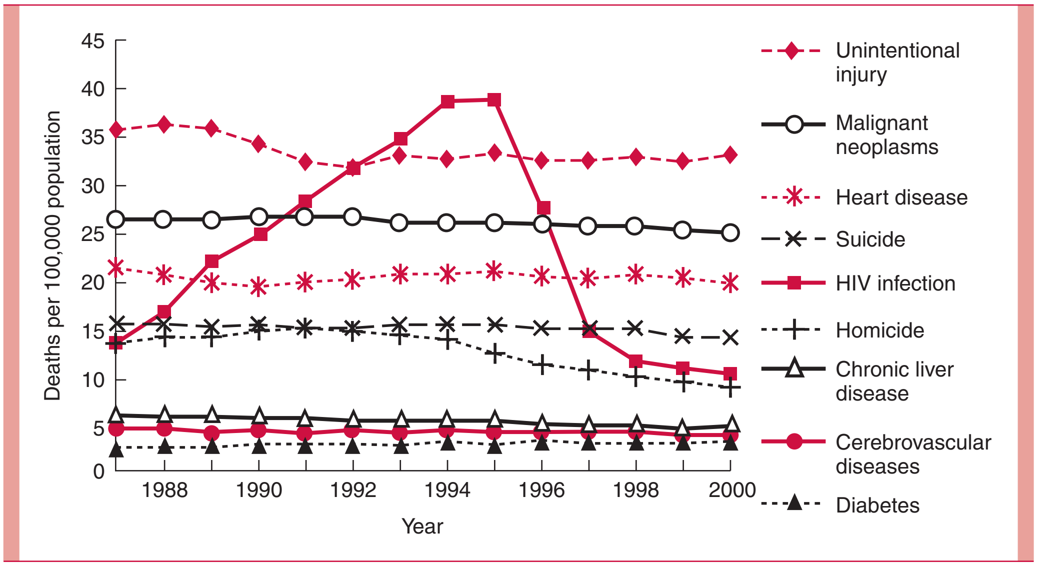



FIGURE 42–6

Death rates per 100,000 population from leading causes of death among persons 25–44 years old, United States, 1987–2000. (*From National Center for Health Statistics, National Vital Statistics System, 2002.*)

TABLE 42–3

### Common Opportunistic Infections in Patients with AIDS

| |
|---|
| **PROTOZOAN** |
| Pneumocystosis (*P. carinii* classification uncertain) |
| Toxoplasmosis |
| *Isospora belli* infection |
| Cryptosporidiosis |
| **FUNGAL** |
| Cryptococcosis |
| Candidiasis |
| Histoplasmosis (disseminated) |
| **MYCOBACTERIAL** |
| Disseminated tuberculosis (especially extrapulmonary) |
| *Mycobacterium avium–intracellulare* complex infections |
| **VIRAL** |
| Persistent mucocutaneous herpes simplex |
| Cytomegalovirus retinitis, gastrointestinal, or disseminated infection |
| Varicella–zoster, persistent or disseminated |
| Progressive multifocal leukoencephalopathy |

As the disease progresses, the number of CD4+ T lymphocytes decline. There is increasing immunodeficiency, and opportunistic infections become more frequent, severe, and difficult to treat. One of the best markers of the severity of AIDS is the absolute number of CD4+ T lymphocytes. Those individuals with overt AIDS almost always have fewer than 400 CD4+ T lymphocytes/mm$^3$ of blood (normal = 800–1200/mm$^3$).

Individuals with overt AIDS usually have fewer than 400 CD4+ lymphocytes/mm$^3$

Patients with full-blown AIDS experience a wide spectrum of infections depending on the severity of their immune defect and on the opportunistic organisms in their normal flora or with which they come in contact (Table 42–3). Some clinical manifestations of AIDS may thus vary by locale. For example, disseminated histoplasmosis is a common complication in the Midwest of the United States, as disseminated toxoplasmosis is in France. These infections are uncommon in areas where the diseases are not endemic. The diversity and anatomic sites of infection vary between patients, and any one patient may have several infections. The most common infection is pneumocystosis, and approximately 50% of AIDS patients who do not receive prophylaxis for pneumocystosis develop *Pneumocystis carinii* pneumonia. In the past, about 25% of all AIDS patients developed Kaposi's sarcoma, but the number of cases has been falling in the United States despite increasing numbers of cases of AIDS. The apparent explanation is that Kaposi's sarcoma is due to a transmitted agent different from HIV, the Kaposi's sarcoma herpesvirus (KSHV); the spread of this organism has diminished as high-risk sexual behavior has decreased especially among homosexual men. Disease due to mycobacteria of the *Mycobacterium avium-intracellulare* complex is common, and AIDS patients are also highly susceptible to *Mycobacterium tuberculosis* infection. Oral thrush and esophagitis due to *Candida albicans* and meningitis due to *Cryptococcus* are commonly encountered fungal infections. Persistent progressive mucocutaneous herpes simplex and herpes zoster infections are common. CMV chorioretinitis is one of the most common opportunistic infections and may result in unilateral or bilateral blindness. Disseminated cytomegalovirus (CMV) infection is also seen and presents with fever and visceral (eg, gastrointestinal) organ involvement.

Pneumocystosis, candidiasis, mycobacteriosis, and CMV are common

CMV retinitis and mycobacterial dissemination usually occur with extremely low CD4+ counts

Specific opportunistic infections are associated with differing levels of CD4+ T-lymphocyte counts. For example, fungal and tuberculous pneumonia may occur with CD4+ T-lymphocyte counts of 200 to 500 cells/mm$^3$, whereas CMV and *M. avium-intracellulare* disease are seen almost exclusively in those whose counts are below 50 to 100 cells/mm$^3$.

HIV is also neurotropic and can lead to dementia

As the duration of survival of AIDS patients became longer due to therapy with the earliest drugs, an increased number developed neurologic manifestations of the disease and lymphoid neoplasms, especially non-Hodgkin's lymphomas. HIV is a neurotropic virus and can be isolated from the cerebrospinal fluid of 50 to 70% of patients. CNS involvement may be asymptomatic, but many patients develop a subacute neurologic illness that produces clinical symptoms varying from mild cognitive dysfunction to severe dementia. Loss of complex cognitive function is usually the first sign of illness. Progression to severe memory loss, depression, seizures, and coma may ensue. Cerebral atrophy involving primarily cortical white matter can be demonstrated by computed tomography or magnetic resonance imaging. Histologically, focal vacuolation of the affected brain tissue with perivascular infiltration of macrophages is noted. Multinucleated giant cells with syncytium formation surround the perivascular infiltrates. Neurologic symptoms do not usually occur until CD4+ T-lymphocyte counts are below 200 cells/mm$^3$.

Aggressive antiviral therapy can slow progression or even result in clinical improvement

Toxicity or mutant resistance limit long-term drug usefulness

The disease spectrum in Africa is similar in many respects to that in the Western world, but many more patients present with severe intractable wasting and diarrhea, known as *slim disease.* Tuberculosis is also more commonly encountered in AIDS patients in Africa, reflecting the higher incidence of the disease in the population in general. The 2-year mortality of AIDS, once the disease has been fully established, was initially 75%, with nearly all persons eventually dying of opportunistic infections or neoplasms. Recent advances in therapy have slowed progression of the disease. Combination therapy, with the inclusion of inhibitors of HIV protease, appears to be responsible for dramatic improvement in many patients, but toxicity or the development of resistance may limit their long-term usefulness. Progression of AIDS and the development of these neurologic manifestations have become less common with the advent of highly active antiretroviral therapy (HAART).

## DIAGNOSIS

EIA screens for antibody

Western blot used for confirmation

The diagnosis of AIDS is most commonly confirmed by demonstrating antibody to the virus or its components. Initial screening tests are performed using whole viral lysates as the target antigens in enzyme immunoassay (EIA) tests. These have a high level of sensitivity, but because false-positive results occur, all positive EIA tests must be confirmed. The confirmatory test is a Western blot analysis, which detects antibodies to specific viral proteins. In this procedure, viral proteins are separated by electrophoresis, transferred to nitrocellulose paper, and incubated with patient sera; antibody bound to the individual proteins is detected by enzyme-labeled anti–human globulin sera (Fig 42–7). Sera from infected patients have antibodies that react with the envelope glycoproteins, core proteins, or both. Tests made with HIV-1 detect antibody in 60 to 90% of patients infected by HIV-2.

Viremia precedes appearance of antibody by 2 to 4 weeks

The combination of EIA and Western blot tests gives a high degree of specificity to test results, but antibody is not detectable by these procedures in the first 2 to 4 weeks after infection. During this period, the individual can still transmit the infection to others by sexual contact or blood donation. Closing this detection gap is particularly important for protection of blood products for transfusion. Although the virus can be grown during this time in mixed lymphocyte cell culture, the methods are impractical and may not be positive for up to 1 month. More practical approaches include nucleic acid–based assays such as the polymerase chain reaction (PCR) for plasma HIV RNA or DNA and the branched chain DNA (bDNA) assay. These are also useful in assessing the benefits of antiviral therapy, as well as in determining if infants born to seropositive mothers are infected or simply demonstrating passively transmitted transplacental antibody.

PCR and bDNA testing used to quantitate plasma viremia and assess drug efficacy

Quantitation of plasma HIV RNA plays an especially important part in management. For example, if a patient's HIV RNA copy number rises during therapy, or fails to fall to low levels (eg, <50 copies/mL), this signals that the antiviral efficacy of the drug regimen

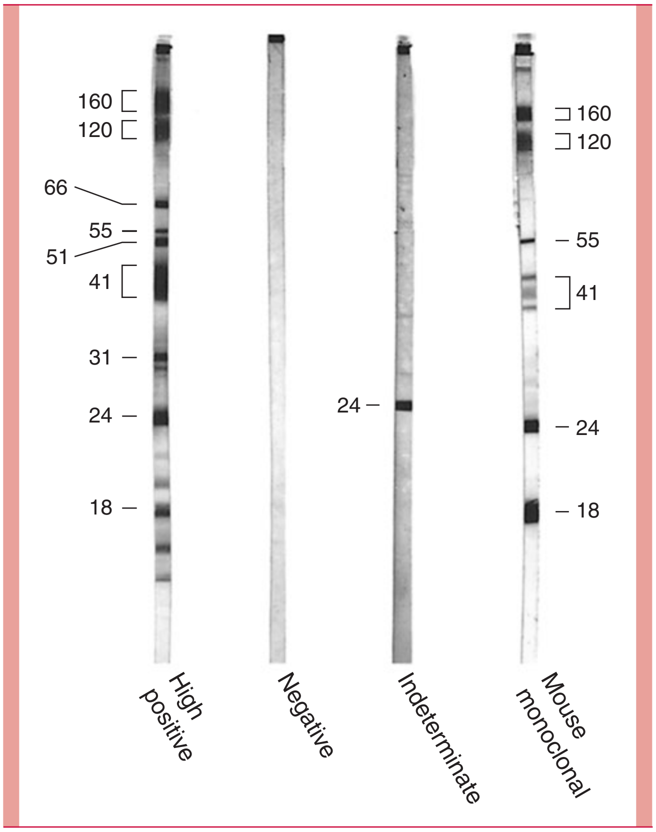



FIGURE 42–7
Western blot detection of HIV-1 antibodies. Note that the "high-positive" serum exhibits antibodies to the HIV-1 envelope glycoproteins of 160, 120 and 41 kilodaltons (kD), to the GAG (core) proteins of 24 and 18 kD, and to other HIV proteins (55 and 51 kD). The "indeterminate" serum exhibits antibody to only the GAG (core) 24-kD protein. The mouse monoclonal blot is a positive control and contains antibodies to key HIV antigens. A positive sample should exhibit antibodies to both envelope and GAG proteins or to both envelope proteins (41 and 120/160 kD).

is inadequate. The most likely explanation is mutational resistance that either preexisted or developed during treatment. Other explanations to be considered include patient noncompliance or inadequate dosing.

## TREATMENT

Initially, only nucleoside inhibitors of HIV reverse transcriptase were available for therapy of HIV infection and they were used singly. Currently, there are at least 16 approved therapeutic agents that inhibit either of two essential viral enzymes: reverse transcriptase or protease. Other antiviral agents under development include those that can block viral entry into the cell, and others that may inhibit viral integrase activity. The characteristics of current representative anti-HIV agents are further summarized in Chapter 13. It is clear that various combinations of these agents are preferable to produce effective virologic and clinical responses.

Combinations of drugs used in treatment

### Initiation of Treatment

Because viral replication proceeds at the phenomenal rate of approximately 10,000 new viruses per day, it seems most rational to begin treatment as soon as infection is detected. However, considerations of toxicity, resistance development, quality of life, cost, and patient wishes are extremely important additional determinants. Although these issues may cause debates regarding early intervention, there is a general consensus that combination therapy should be initiated when CD4+ count falls below 500/mm$^3$ or the plasma HIV viral load is more than 5000 copies/mm$^3$ of viral RNA.

Decision to treat aggressively is influenced by CD4+ count and viral load

### Resistance

RNA viruses tend to have frequent mutations, and the genome of HIV is highly variable. This results in part from the extremely high turnover rate of virions per day. As a result, resistance to an antiviral is a regular and often rapid development. Use of antiviral therapies

Drug resistance is expected development with treatment that maximally suppress HIV viral load appear to diminish the appearance of resistant virus.

Prophylaxis of opportunistic infections is especially important

In addition to the primary treatment of HIV, patients with CD4+ counts of less than 200/mm$^3$ should begin prophylactic regimens to prevent *P. carinii* pneumonia; when CD4+ counts are less than 75 to 100/mm$^3$ they should receive prophylaxis for mycobacterial and fungal infection.

## PREVENTION

Education is the cornerstone of prevention

Screening for asymptomatic infection in pregnancy aids effective prophylaxis

The spread of AIDS has been facilitated by changing sexual mores, injection drug use, and, in some parts of the world, disruption of family and tribal units as a consequence of industrialization and urbanization. These factors are obviously not subject to rapid change. Immediate prevention must be based on education about the means of transmission and easy access to condoms and safe needles for those large numbers of people who continue to place themselves at risk. The epidemiologic and laboratory methods used to control foci of other major epidemic diseases pose particular problems in AIDS control at present. Quite apart from questions of potential discrimination against infected individuals and the calamitous effects of false-positive serologic test results, the sheer magnitude and cost of case finding and contact tracing at present limit this approach. Detection and treatment of HIV-infected pregnant women has also been shown to be effective in reducing perinatal infection.

Caesarian section, particularly elective rather than emergent, is also a preventive, as is the avoidance of breast feeding by HIV positive mothers. Much research is underway to develop vaccines against the virus, but the marked mutability of HIV greatly complicates this approach. Furthermore, passage of virus between fused cells and in syncytia protects it from antibody neutralization in established disease. The search continues for conserved epitopes of the surface glycopeptides that might provide possible antigenic targets. Antiviral treatment utilizing combinations of agents may prevent infection of accidentally exposed individuals (eg, health care workers). This therapy must be initiated within hours of an accident if it is to have any chance of success.

## ADDITIONAL READING

Guidelines for using antiretroviral agents among HIV-infected adults and adolescents: Recommendations of the panel on clinical practices for the treatment of HIV. *MMWR* 2002;51:RR-7. Current guidelines and their rationale are periodically summarized. (Also available with updates on www.cdc.gov/mmwr.)

Levy JA. *HIV and the Pathogenesis of AIDS,* 2nd ed. Washington, D.C.: ASM Press; 1998. A current description of HIV and its pathogenic potential.

Mellors JW, Kingsley LA, Rinaldo CR Jr, et al. Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. *Ann Intern Med* 1995;122:573–579. This illustrates how progression of HIV infection can be predicted based on viral load data.

# CHAPTER 43

# Papovaviruses

W. Lawrence Drew

The papovaviruses of medical interest include the papillomaviruses and polyomaviruses .

## Papillomaviruses

### VIROLOGY

Papillomaviruses are small, unenveloped, double-stranded DNA viruses exhibiting cubic symmetry. About 55 nm in diameter, they cause epidermal papillomas and warts in a wide range of higher vertebrates. Different members of the group are generally species specific. For example, bovine and human papillomaviruses infect only the hosts reflected in their names. In some cases, tumors caused by these agents can become malignant and the role of these agents as causes of certain human cancers is being clarified. Papillomaviruses have not been grown in tissue culture, and most of the virologic information has derived from molecular studies.

Naked, double-stranded DNA viruses

Have not been grown in vitro

The genomes of many of the papillomaviruses have now been cloned and compared by restriction endonuclease and DNA homology procedures (see Chapters 4 and 15). These studies have shown a wide genomic diversity among papillomaviruses that infect different species and also among those that infect humans. This has led to the allocation of numbers for the different genotypes.

Great genomic diversity

### PAPILLOMAVIRUS DISEASE

Clinical Capsule

More than 70 genotypes of human papillomaviruses (HPVs) have been identified in human specimens. Some of the genotypes are antigenically (phenotypically) different, and groups of genotypes are associated with specific lesions. HPVs have been identified in plantar warts; in flat and papillomatous warts of other skin areas; in juvenile laryngeal papillomas; and in a variety of genital hyperplastic epithelial lesions, including cervical, vulvar, and penile warts and papillomas. In addition, they are associated with premalignant (cervical intraepithelial neoplasia) and malignant disease (cervical cancer). Lesions comparable to those occurring in the cervix are now recognized in the anus, especially among men who have sex with men and are infected by human immunodeficiency virus, or HIV.

## EPIDEMIOLOGY

Cutaneous nongenital warts usually occur in children and young adults; presumably immunity to the HPV genotypes causing these lesions develops and appears to provide protection. Twelve HPV genotypes have been identified in genital lesions of humans, and there are many apparently silent infections with these viruses. Cross immunity does not occur, and sequential infection with multiple genotypes does take place. The incidence of HPV infections has almost certainly been increasing, and they may now constitute the most common sexually transmitted disease. From 20 to 60% of adult women in the United States are infected with one or another of the genotypes. HPV types 6 and 11 are associated most commonly with benign genital warts in males and females and with some cellular dysplasias of the cervical epithelium, but these lesions rarely become malignant. They can be perinatally transmitted and cause infantile laryngeal papillomas. Types 16, 18, 31 and 45 may also cause warty lesions of the vulva, cervix, and penis. Infections with these viral types, especially 16, may progress to malignancy. Viral genomes of these four types are found in a proportion of markedly dysplastic uterine cervical cells, in carcinoma in situ, and in cells of frankly malignant lesions. Human papillomavirus infection is now considered to be a cause of the majority of carcinomas of the cervix. Papillomavirus infection of the anus is a clinical problem in homosexual men, especially those with acquired immunodeficiency syndrome (AIDS), and it appears to be related to the subsequent development of anal neoplasia in these individuals.

HPV types 6 and 11 common; rarely lead to malignancy

Types 16, 18, 31, and 45 associated with dysplasia and malignancy

## PATHOGENESIS

Papillomaviruses have a predilection for infection at the junction of squamous and columnar epithelium (eg, in the cervix and anus). Papillomaviruses were the first DNA viruses linked to malignant changes. In the mid-1930s, Shope demonstrated that benign rabbit papillomas were due to filterable agents and could advance to become malignant squamous cell carcinomas. External cofactors, such as coal tar, could hasten this process. However, work on the biology and mechanism by which these agents foster malignant transformation has been impeded by the inability to cultivate papillomaviruses in vitro. Molecular probes to detect viral products in vivo indicate that replication and assembly of these viruses take place only in the differentiating layers of squamous epithelia, a situation that has not been reproduced in vitro.

Replication in squamous epithelium

The first evidence that HPVs could be associated with human malignant disease came from observations on epidermodysplasia verruciformis. This disease has a genetic basis that results in unusual susceptibility to HPV types 5 and 8, which produce multiple flat warts. About one third of affected patients develop squamous cell carcinoma from these lesions.

The mechanism of oncogenicity of HPV is less clear. Cells infected with genomes of several papillomaviruses can transform cells and produce tumors when injected into nude (T lymphocyte–deficient) mice. The viral genome exists as multiple copies of a circular episome within the nucleus of transformed cells but is not integrated into the cellular genome. This appears also to be the case with benign human lesions. In malignant tumors, part of the viral genome is found integrated into the cellular genome, but integration is not site specific. Both the integrated viral genome and the extrachromosomal form carry their own transforming genes. Host cells normally produce a protein that inhibits expression of papillomavirus transforming genes, but this can be inactivated by products of the virus and possibly by other infecting viruses, thus allowing malignant transformation to occur. HPV DNA is found in more than 95% of cervical carcinoma specimens when tested by polymerase chain reaction (PCR).

Viral genomes carry their own transforming genes

# PAPILLOMAVIRUS: CLINICAL ASPECTS

## MANIFESTATIONS

Cutaneous warts can vary from flat to deep plantar growths. Although they can persist for years, they ultimately spontaneously regress. Respiratory papillomatosis due most often to types 6 and 11 occurs as intraoral or laryngeal lesions. These tend to occur in infants as a result of natal exposure, or in full grown adults.

Oral or laryngeal papillomatosis in infants infected during delivery

External genital HPV infection occurs as exophytic genital warts (condyloma acuminata) caused most often by types 6 or 11. Lesions may increase in size to cauliflower-like appearance during pregnancy or immunosuppression. Genital HPV infection is most often benign, and many lesions reverse spontaneously. However they may become dysplastic and proceed through a continuum of cervical intraepithelial neoplasm to severe dysplasia and/or carcinoma in situ. The most common HPV in the malignant lesions is type 16, although this genotype, as well as the others, is most apt to cause lesions that regress spontaneously. Higher grade malignancy is most apt to occur in the cervix, but the rate of anal carcinoma related to HPV appears to be increasing, especially in AIDS patients.

Anal carcinoma due to HPV may be increasing

## DIAGNOSIS

HPV does not grow in routine tissue culture, and antibody tests are rarely used. Papillomavirus infection leads to perinuclear cytoplasmic vacuolization and nuclear enlargement, referred to as poikilocytosis, in epithelial cells of the cervix or vagina. These changes can be seen in a routine Papanicolaou smear. The use of immunoassays to detect viral antigen and in situ hybridization or PCR to detect specific viral DNA in cervical swabs or tissue is more sensitive (Fig 43–1), but the clinical utility of detecting specific HPV types in clinical specimens remains to be determined. Detection of an abnormal cytology due to HPV should prompt colposcopy to assist in following or treating patients with abnormal lesions.

Poikilocytosis can be seen in cytologic specimens

Molecular methods to detect specific genotypes in biopsies of cervical swabs are available

## TREATMENT AND PREVENTION

Current treatment of HPV is usually either cytotoxic or surgical. Among the topical cytotoxins are podophyllin, podophyllotoxin, 5-fluorouracil, and trichloroacetic acid.

Recurrences are common after topical treatment

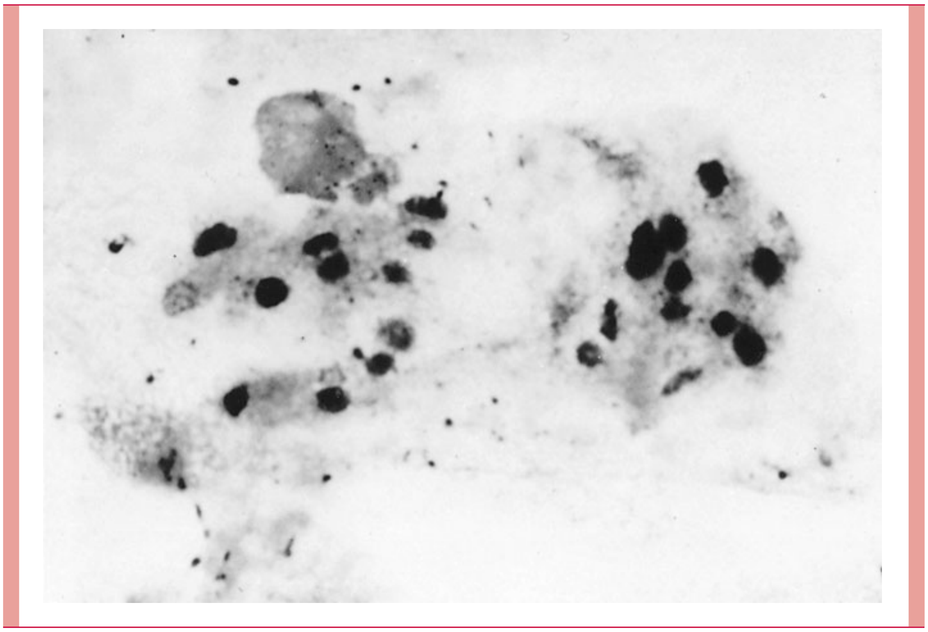


FIGURE 43–1 Human papillomavirus (HPV) type 16 DNA demonstrated in a cervical smear by in situ hybridization. The dark dots represent detection of HPV DNA sequences by the DNA probe.

Recurrences are common following cessation of treatment because of survival of virus in the basal layers of the epithelium. Systemic and local interferon therapy has shown some promise as a treatment, although lesions tend to recur after cessation of therapy. Cervical lesions may be treated with electrocautery.

Future prospects for prevention by vaccines

A recent large, prospective study of a HPV-16 virus-like-particle vaccine (an L1 polypeptide expressed in yeast) conducted among women 16 to 23 years of age indicated the potential for prevention of persistent infection, at least with this type, is high.

# POLYOMAVIRUSES

## VIROLOGY

Naked DNA viruses

Can transform cells in vitro

The polyomaviruses include the JC virus (JCV) and BK virus (BKV) of humans and simian virus 40 (SV40) of monkeys. Polyomaviruses, like papillomaviruses, are members of the papovavirus family. They are also double-stranded, naked capsid DNA viruses and are widely distributed among various animal species, usually without causing apparent disease. However, they are able to transform cells of a variety of heterologous cell lines in culture.

## POLYOMAVIRUS DISEASE

**CLINICAL CAPSULE**

Polyomaviruses are closely related to papillomaviruses but are not known to cause clinical disease in immunocompetent patients. They can cause progressive multifocal leukoencephalopathy (PML) and hemorrhagic cystitis/nephropathy in immunocompromised patients.

### EPIDEMIOLOGY

Latency is common

Disease associated with immunocompromise

Approximately 80% of adults show serologic evidence of JCV and BKV infection with no known clinical manifestations, but the viruses remain latent and may reactivate and cause disease in immunocompromised patients. BKV is estimated to cause renal disease in 2 to 5% of renal transplant recipients.

### PATHOGENESIS

Do not cause malignancies in their natural hosts

Polyomaviruses can produce malignant tumors in certain experimental animals but, interestingly, not in their natural hosts. For example, SV40 can produce lymphocytic leukemia and a variety of reticuloendothelial cell sarcomas in baby hamsters but is not oncogenic in its natural monkey host. Fortunately, even though it can transform some human cells in vitro, it fails to produce disease in humans, a fact that became apparent on follow-up of recipients of early batches of poliomyelitis vaccine produced in monkey kidney cell cultures that were contaminated with live SV40.

Interact with cells in a variety of ways

The reason polyomaviruses fail to produce tumors in their natural hosts is uncertain, but it may be due to the fact that the viruses are usually cytocidal under these conditions. From a biological point of view, the polyomaviruses are particularly useful models of oncogenicity because they can be readily studied in vitro and interact with cells in different ways. In some, they produce lytic infections and cell death with production of complete virions. In others, they integrate randomly into the cell genome and cause transformation by the expression of one or more of the viral genes. No human tumor has been shown to be caused by polyomaviruses.

# POLYOMAVIRUSES: CLINICAL ASPECTS

## MANIFESTATIONS

### Progressive Multifocal Leukoencephalopathy

PML is a rare, subacute, degenerative disease of the brain found primarily in adults with other chronic diseases, especially AIDS and reticuloendothelial malignancies, or those receiving immunosuppressive agents. The disease is characterized by the development of impaired memory, confusion, and disorientation, followed by a multiplicity of neurologic symptoms and signs that include hemiparesis, visual disturbances, incoordination, seizures, and visual abnormalities. PML is progressive, with death usually occurring 3 to 6 months after onset of symptoms. The incidence of PML has increased concomitantly with the AIDS epidemic.

PML is a degenerative, progressive brain disease

In PML, cerebrospinal fluid (CSF) findings are often normal, although some patients show a slight increase in lymphocytes, and protein levels may be elevated. Pathologically, foci of demyelination are found, surrounded by giant, bizarre astrocytes containing intranuclear inclusions. The demyelination is due to viral damage to oligodendroglial cells, which synthesize and maintain myelin. Abundant JCV particles can be seen in the brain by electron microscopy (Fig 43–2) and may be concentrated within the nuclei of oligodendrocytes. JCV DNA sequences have been demonstrated by PCR in the brain of patients without PML or demyelinating lesions, suggesting that the virus may be latent in the brain prior to immunosuppression. There is no specific treatment for PML, although reducing the immunosuppression, if possible, may have some clinical benefit.

JCV in cell nuclei, with demyelination

No specific treatment

### Urinary Tract Infection

Infection of the urinary tract with JCV and BKV can be demonstrated frequently in immunocompromised patients but usually in those without symptoms or evidence of renal injury. BKV is associated with a hemorrhagic cystitis, particularly in bone marrow and renal transplant recipients. In addition, BKV is also the cause of a severe nephropathy and

BKV causes hemorrhagic cystitis and nephritis

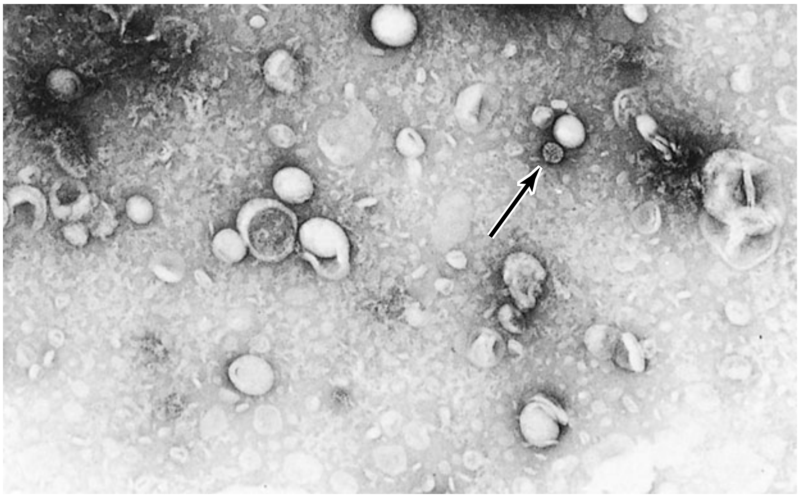


FIGURE 43–2
JC virus (arrow) among debris of cells from a brain biopsy of a case of progressive multifocal leukoencephalopathy. (*Reprinted with permission from Palmer E, Martin ML.* An Atlas of Mammalian Viruses. *Boca Raton, FL: CRC Press; 1982. Copyright 1982 by CRC Press, Inc.*)

vasculopathy that may lead to kidney loss in renal transplant recipients. The disease develops months after renal transplantation. Treatment consists of reducing immunosuppression, but as many as 50% of patients with this syndrome may require nephrectomy.

## DIAGNOSIS

BKV can be isolated in cell culture

Both viruses can be detected by PCR

Urine from patients excreting these polyomaviruses may contain "decoy" cells similar to those from patients excreting cytomegalovirus. The nucleus of cytomegalovirus-infected cells is smaller with a larger halo effect, that is, a clear zone around the inclusion but within the nuclear membrane. The brain oligodendrocytes exhibit similar changes in patients with PML. BKV can be isolated by routine culture in diploid fibroblast or Vero monkey kidney cells, but nephropathy is preceded by plasma PCR positivity. At present, a kidney biopsy is required for definitive diagnosis. Viral antigens can be demonstrated in tissue by a variety of immunoassays. JCV DNA has been demonstrated in the brain of PML patients by PCR, and PCR of CSF is becoming the diagnostic test for PML.

## ADDITIONAL READING

Bauer HM, Ting V, Greer CE, et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. *JAMA* 1991;265:472–477. Good report on the epidemiology of human papillomaviruses and means of diagnosis.

Koustky LA, Ault KA, Wheeler CM, et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002;347:1645–1651. This is a landmark trial, indicating it may indeed be possible to reduce the incidence of cervical cancer with vaccines. Administration of this HPV-16 vaccine reduced the incidence of both HPV-16 infection and HPV-16 related cervical intraepithelial neoplasia. Immunizing HPV-16-negative women may eventually reduce the incidence of cervical cancer.

Major EO, Ault GS. Progressive multifocal leukoencephalopathy: Clinical and laboratory observations in a viral induced demyelinating disease in the immunodeficient patient. *Curr Opin Neurol* 1995;8:184. An excellent review of papovavirus CNS disease.

Palefsky JM. Human papillomavirus infection among HIV-infected individuals. *Hematol Oncol Clin North Am* 1991;5:357–370. Provides guidelines for the management of cervical and anal neoplasia in HIV-infected persons.

Petrogiannis-Haliotis T, Sakoulas G, Kirby J, et al. BK-related polyomavirus vasculopathy in a renal transplant recipient. *N Engl J Med* 2001;345:1250–1255. This is an extremely well-executed case study, demonstrating current diagnostic approaches to human polyomavirus infections, and the virus tropism for vascular endothelial cells.

CHAPTER 44

# Persistent Viral Infections of the Central Nervous System

W. Lawrence Drew

Evidence has accumulated during the past 30 years that a variety of progressive neurologic diseases in both animals and humans are caused by viral or other filterable agents that share some of the properties of viruses (Table 44–1). These illnesses have been termed "slow viral diseases" because of the protracted period between infection and the onset of disease as well as the prolonged course of the illness, but a better term is "persistent viral infection."

Progressive neurologic diseases

Most persistent viral infections involve well-differentiated cells, such as lymphocytes and neuronal cells. They can be classified as (1) diseases associated with "conventional" viral agents that possess nucleic acid genomes and protein capsids, induce immune responses, and can be grown in cell culture systems; and (2) diseases associated with "unconventional" viruses that are small, filterable infectious agents, known as "prions," which are transmissible to certain experimental animals, but that do not contain nucleic acids, do not appear to be associated with immune or inflammatory responses by the host and have not been cultivated in cell culture.

Include conventional viruses and unconventional agents

Do not produce immune or inflammatory responses

Persistence of conventional viruses can result from integration of viral nucleic acid into the host genome, mutations that interfere with or severely limit viral replication or antigenicity, failure of host immune systems to recognize virus or infected cells, or perhaps by encoding of the causative agent itself into the normal host cell genome.

Can be due to a variety of mechanisms

## DISEASES ASSOCIATED WITH CONVENTIONAL AGENTS

The following conditions are the major persistent infections caused by conventional viral agents. They are summarized in Table 44–1.

### Subacute Sclerosing Panencephalitis

Subacute sclerosing panencephalitis is considered in Chapter 34. It is a rare chronic measles virus infection of children that produces progressive neurologic disease characterized by an insidious onset of personality change, progressive intellectual deterioration, and both motor and autonomic nervous system dysfunctions.

Persistence of measles virus after acute childhood infection

TABLE 44–1

**Conventional Viruses Causing Persistent Central Nervous System Infections**

| Disease | Agent |
|---|---|
| Subacute sclerosing panencephalitis | Measles virus |
| Progressive panencephalitis following congenital rubella | Rubella virus |
| Progressive multifocal encephalopathy | Papovavirus (JC) |
| AIDS dementia complex | Human immunodeficiency virus |
| Persistent enterovirus infection of the immunodeficient | Enteroviruses |

*Abbreviations:* AIDS, acquired immunodeficiency syndrome.

## Progressive Postrubella Panencephalitis

Can be a late sequela of congenital rubella infection

Even more rarely, a degenerative neurologic disorder similar to subacute sclerosing panencephalitis may be related to persistent rubella virus infection of the central nervous system (CNS). This condition is seen most often in adolescents who have had the congenital rubella syndrome. Rubella virus has been isolated from brain tissue in these patients using cocultivation techniques.

## Progressive Multifocal Leukoencephalopathy

Progressive neurologic disease of severely immunocompromised persons

Progressive multifocal leukoencephalopathy (PML) is a subacute, degenerative disease of the brain found primarily in adults with (1) immunosuppressive diseases, especially acquired immunodeficiency syndrome (AIDS) and reticuloendothelial malignancies; or (2) diseases requiring therapy with immunosuppressive agents. PML is due to a papovavirus and is considered in Chapter 43.

## Persistent Enterovirus Infection

Associated with humoral immunodeficiencies

Temporary improvement with hyperimmune globulin

Persons with congenital or severe acquired immunodeficiency, especially those with agammaglobulinemia, may develop a chronic CNS infection due to an echovirus or other enterovirus. Headache, confusion, lethargy, seizures, and cerebrospinal fluid (CSF) pleocytosis are common manifestations. The virus can be isolated from the CSF. Clinical improvement may be achieved by the administration of human hyperimmune globulin to the infecting virus type. Relapse, however, occurs if therapy is discontinued, indicating persistence of virus despite the therapy.

## AIDS Dementia Complex

Late stages of AIDS

Human immunodeficiency virus causes a persistent infection of the CNS in many patients with symptomatic AIDS. The clinical course may vary from a mild subacute illness to severe progressive dementia (see Chapter 42).

# HUMAN DISEASES CAUSED BY UNCONVENTIONAL VIRAL AGENTS: SUBACUTE SPONGIFORM ENCEPHALOPATHIES

A group of progressive degenerative diseases of the CNS has been shown to be caused by infectious agents with unusual physical and chemical properties, which are now known as prions. The Nobel prize in Medicine for 1997 was awarded to Stanley Prusiner for his work in identifying the role of prions in disease. Prions cause bovine spongiform encephalopathy in cattle, scrapie in sheep, and five fatal CNS diseases in humans

TABLE 44–2

**Unconventional Virus (Prion) Diseases[a]**

| Humans | Animals (Primary hosts) |
|---|---|
| Creutzfeldt-Jakob disease[b] | Scrapie (sheep) |
| Variant Creutzfeldt-Jakob disease | Transmissible mink encephalopathy (mink) |
| Gerstmann-Straüssler-Scheinker syndrome | Chronic wasting disease (mule deer, elk) |
| Kuru | Bovine spongiform encephalopathy (cows)[b] |
| Fatal familial insomnia | |

[a] Subacute spongiform encephalopathies.
[b] Prion agents of variant Creutzfeldt-Jakob disease and bovine spongiform encephalopathy are identical.

(Table 44–2). Prions can be the etiologic agents of inherited, communicable, or sporadic diseases. The pathogenesis of these illnesses is not well understood, but the pathologic and clinical features are similar. Varying degrees of neuronal loss and astrocyte proliferation occur. The diseases are known as "spongiform" encephalopathies because of the vacuolar changes in the cortex and cerebellum. The incubation periods of these diseases are months to years, and their courses are protracted and inevitably fatal.

Prions affect animals and humans

Cause neuronal loss and spongiform changes in brain

A prion is defined as a "small proteinaceous infectious particle" that is not inactivated by procedures that destroy nucleic acids (Table 44–3). They are small with diameters of 5–100 nm or less, produce characteristic infections, and can remain viable even in formalinized brain tissue for many years. They are resistant to ionizing radiation, boiling, and many common disinfectants. Recognizable virions have not been found in tissues by electron microscopy, and the agents have not been grown in cell culture.

Infectious agents resist inactivation

Nucleic acids absent

A prion is composed of proteins encoded by a normal cellular gene. The protein, designated PrPc, is converted from a normal benign form into a disease-causing form by a change in conformation to a protein designated PrPsc (for the scrapie protein). Brain extracts from scrapie-infected animals contain PrPsc, which is not found in the brains of normal animals; PrPsc is the prion that is responsible for transmission and infection. The conformational change is also the way that prions multiply; that is, contact with PrPsc results in a conformational change of the normal host cell protein PrPc and the formation of additional PrPsc. Proliferation of PrPsc prions and the consequent pathology results from this process. During scrapie infection, prion protein may aggregate into birefringent rods

PrPc is encoded by a normal cellular gene

Conformational change to PrPsc results in disease and prion proliferation

TABLE 44–3

**Biologic and Physical Properties of Prions**

- Chronic progressive pathology without remission or recovery
- No inflammatory response
- No alteration in pathogenesis by immunosuppression or immunopotentiation
- Estimated diameter of 5–100 nm
- No virion-like structures visible by electron microscopy
- Replication to high titers in susceptible tissue
- Transmissible to experimental animals
- No interferon production or interference by conventional viruses
- Unusual resistance to ultraviolet irradiation, alcohol, formalin, boiling, proteases, and nucleases
- Can be inactivated by prolonged exposure to steam autoclaving or 1N or 2N NaOH

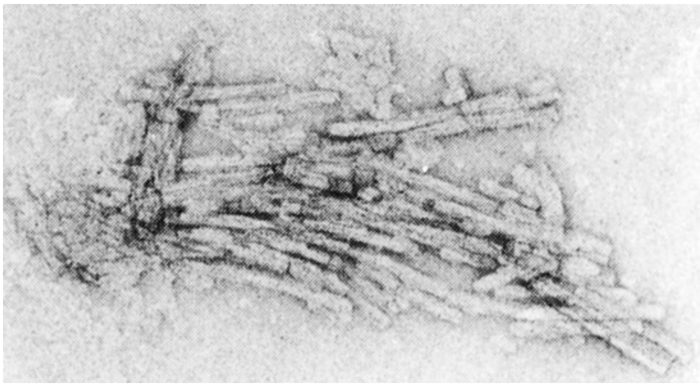


FIGURE 44–1
Amyloid-like fibrils (scrapie-associated fibrils) observed in brain extract of a patient with Creutzfeldt–Jakob disease. (*Reprinted with permission from Bockman JM, Kingsbury DT, McKinley MP, et al. Creutzfeldt–Jakob disease prion proteins in human brains.* N Engl J Med *1985;312:73–82.*)

and form filamentous structures termed scrapie-associated fibrils (Fig 44–1), which are found in membranes of scrapie-infected brain tissues.

## Kuru

Women and children of the Fore people of New Guinea

Transmissible to primates

Associated with cannibalism

Kuru was a subacute, progressive neurologic disease of the Fore people of the Eastern Highlands of New Guinea. The disease was brought to the attention of the Western world by Gadjusek and Zigas in 1957. Although the illness was localized and decreasing in incidence, its study has thrown light on the transmissibility and infectious nature of similar encephalopathies. Epidemiologic studies indicated that kuru usually afflicted adult women, or children of either sex. The disease was rarely observed outside of the Fore region, and outsiders in the region did not contract the disease. The symptoms and signs were ataxia, hyperreflexia, and spasticity, which led to progressive dementia, starvation, and death. Pathologic examination revealed changes only in the CNS, with diffuse neuronal degeneration and spongiform changes of the cerebral cortex and basal ganglia. No inflammatory response was apparent. Inoculation of infectious brain tissue into primates produced a disease that caused similar neurologic symptoms and pathologic manifestations after an incubation period of approximately 40 months. Epidemiologic studies indicated that transmission of the disease in humans was associated with ingestion of a soup made from the brains of dead relatives and eaten in honor of the deceased. Clinical disease developed 4 to 20 years after exposure. Since the elimination of cannibalism from the Fore culture, kuru has disappeared.

## Creutzfeldt–Jakob Disease

Progressive disease, usually occurring among elderly

Creutzfeldt–Jakob disease is a progressive, fatal illness of the CNS that is seen most frequently in the sixth and seventh decades of life. The initial clinical manifestations are a change in cerebral function, usually diagnosed initially as a psychiatric disorder. Forgetfulness and disorientation progress to overt dementia and the development of changes in gait, increased tone in the limbs, involuntary movement, and seizures. These manifestations resemble those of kuru. The disorder usually runs a course of 4–7 months, eventually leading to paralysis, wasting, pneumonia, and death.

Pathology identical to kuru

Transmission to animals

Creutzfeldt–Jakob disease is found worldwide, with an incidence of disease of one case per million per year. The mode of acquisition is unknown, but it occurs both sporadically (85%) and in a familial pattern (15%). Infection has also been transmitted by dura mater grafts, corneal transplants, by contact with contaminated electrodes or instruments used in neurosurgical procedures, and by pituitary-derived human growth hormone. The latter was responsible for more than 100 cases. The incubation period of the disease is approximately 3 to greater than 20 years.

The pathology of Creutzfeldt–Jakob disease is identical to that of kuru. It has been transmitted to chimpanzees, mice, and guinea pigs by inoculation of infected brain tissue, leukocytes, and certain organs. High levels of infectious agent have been found, especially in the brain, where they may reach $10^{-7}$ infectious doses per gram of brain tissue. Nonpercutaneous transmission of disease has not been observed, and there is no evidence of transmission by direct contact or airborne spread.

Brains from patients with Creutzfeldt–Jakob disease have the birefringent rods and fibrillar structures noted in scrapie (see Fig 44–1). Identification of PrPsc and antibodies directed against it may become a useful diagnostic adjunct to neuropathologic examination of brain tissue. Pathologic examination of brain tissue is the only definitive diagnostic test.

Scrapie-like structures seen in brain

There is no effective therapy for Creutzfeldt–Jakob disease, and all cases have been fatal. The small risk of nosocomial infection is related only to direct contact with infected tissue. Stereotactic neurosurgical equipment, especially that used in patients with undiagnosed dementia, should not be reused. In addition, organs from patients with undiagnosed neurologic disease should not be used for transplants. Growth hormone from human tissue has now been replaced by a recombinant genetically engineered product. The agent of Creutzfeldt–Jakob disease has not been transmitted to animals by inoculation of body secretions, and no increased risk of disease has been noted in family members or medical personnel caring for patients. Recommendations for disinfection of potentially infectious material include treatment for 1 hour with 2 N NaOH or by autoclaving at 132°C for 60–90 minutes. Others recommend even more extensive treatment such as combining these two procedures to ensure inactivation.

Nosocomial infections preventable by avoidance of potentially infectious materials, careful sterilization

## Gerstmann-Straüssler-Scheinker Disease

Gerstmann-Straüssler-Scheinker disease is similar to Creutzfeldt–Jakob disease but occurs at a younger age (fourth to fifth decade). Cerebellar ataxia and paralysis are common, but dementia is less often seen. The disease evolves over several years. It was originally thought to be familial but also occurs sporadically, very rarely.

Gerstmann-Straüssler-Scheinker disease similar to Creutzfeldt–Jakob disease but evolves more slowly

## Fatal Familial Insomnia

This is a recently recognized familial prion disease in which a syndrome of sleeping difficulty is followed by progressive dementia. It occurs in patients aged 35 to 61, culminating in death within 13 to 25 months. The infectious agent has been transmitted to experimental animals.

Sleeping difficulties progressing to dementia

## Bovine Spongiform Encephalopathy ("Mad Cow Disease") and "Variant Creutzfeldt–Jakob Disease"

Bovine spongiform encephalopathy (BSE) was identified in 1986, after it began striking cows in the United Kingdom, causing them to become uncoordinated and unusually apprehensive. The source of the emerging epidemic was soon traced to a food supplement that included meat and bone meal from dead sheep. The methods for processing sheep carcasses had been changed in the late 1970s. Once they would have eliminated the "scrapie agent" in the supplement, but now they apparently did not.

To combat BSE, the British government banned the use of animal-derived feed supplements in 1988, and the epidemic among cattle, which peaked at nearly 40,000 cases in 1992, decreased to less than 4000 new cases in 1997. By February 2002, most European countries had reported cases of BSE but new infections have largely ceased as a result of imposing tight controls on cattle feed. The United States has been spared, as measured by over 19,000 cattle brain examinations. The incubation period in cattle was determined to be 2 to 8 years. In addition to the incoordination and apprehension, the cows exhibited hyperesthesia, hyperreflexia, muscle fasciculations, tremors, and weight loss. Autonomic dysfunction was frequently manifested as reduced rumination, bradycardia and other cardiac arrhythmias.

Source was meat and bone meal from sheep in cattle feed

Unfortunately, the prion that causes BSE survived the heat of cooking and was transmitted to humans who inadvertently consumed infected bovine neural tissue or bone marrow (both are sometimes found in processed meats, depending on the rendering procedures used). To date, over 100 humans with "variant Creutzfeldt–Jakob disease," have

Variant Creutzfeldt–Jakob disease apparently transmitted by infected bovine tissues to humans died in the United Kingdom. The cases frequently present in young adults as psychiatric problems progressing to neurologic changes and dementia, with death in an average of 14 months. It appears that destruction of diseased cattle and the changes in livestock feeds have prevented further cases.

## ADDITIONAL READING

Almond J, Pattison J. Human BSE. *Nature* 1997;389:437–438. This article reviews evidence for transmission of BSE to humans, especially molecular identity of proteins from cattle and human brains.

Hill AF, Desbruslais M, Joiner S, et al. The same prion strain causes vCJD and BSE. *Nature* 1997;389:448–450. Summarizes evidence that the new variant Creutzfeldt–Jakob disease is caused by the same prion that causes BSE.

Johnson RT, Gibbs CJ Jr. Creutzfeldt-Jakob disease and related transmissible spongiform encephalopathies. *N Engl J Med* 1998;339:1994–2004. A review of prion-caused diseases, their clinical manifestations, and their diagnosis.

Prusiner SB. Molecular biology and pathogenesis of prion diseases. *Trends Biochem Sci* 1997;21:482–487. This article explains reproduction of prions and their neuropathologic potential.

# PART VII

# PATHOGENIC FUNGI

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CHAPTER 45

# Characteristics of Fungi

KENNETH J. RYAN

Fungi are a distinct class of microorganisms, most of which are free-living in nature where they function as decomposers in the energy cycle. Of the more than 200,000 known species, fewer than 200 have been reported to produce disease in humans. These diseases, the mycoses, have unique clinical and microbiologic features and are increasing in immunocompromised patients.

## GENERAL NATURE OF FUNGI

Fungi are eukaryotes with a higher level of biological complexity than bacteria. They may be unicellular or may differentiate and become multicellular by the development of branching filaments. They reproduce sexually or asexually. The mycoses vary greatly in their manifestations but tend to be subacute to chronic with indolent, relapsing features. Acute disease, such as that produced by many viruses and bacteria, is uncommon with fungal infections.

Cell organization is eukaryotic

### STRUCTURE

The fungal cell has typical eukaryotic features, including a nucleus with a nucleolus, nuclear membrane, and linear chromosomes. The cytoplasm contains an actin cytoskeleton and organelles, such as mitochondria and the Golgi apparatus. Fungal cells, which have a rigid cell wall external to the cytoplasmic membrane, differ from mammalian cells. The composition of that wall makes fungi different from bacteria and plants. Another important difference from mammalian cells involves the sterol makeup of the cytoplasmic membrane. In fungi, the dominant sterol is ergosterol; in mammalian cells, it is cholesterol. Fungi are usually in the haploid state, although diploid nuclei are formed through nuclear fusion in the process of sexual reproduction.

Presence of a nucleus, mitochondria, and endoplasmic reticulum

Ergosterol, not cholesterol, makes up cell membrane

The chemical structure of the cell wall in fungi is markedly different from that of bacterial cells in that it does not contain peptidoglycan, glycerol or ribitol teichoic acids, or lipopolysaccharide. In their place are the polysaccharides **mannan, glucan,** and **chitin** in close association with each other and with structural proteins (Fig 45–1).

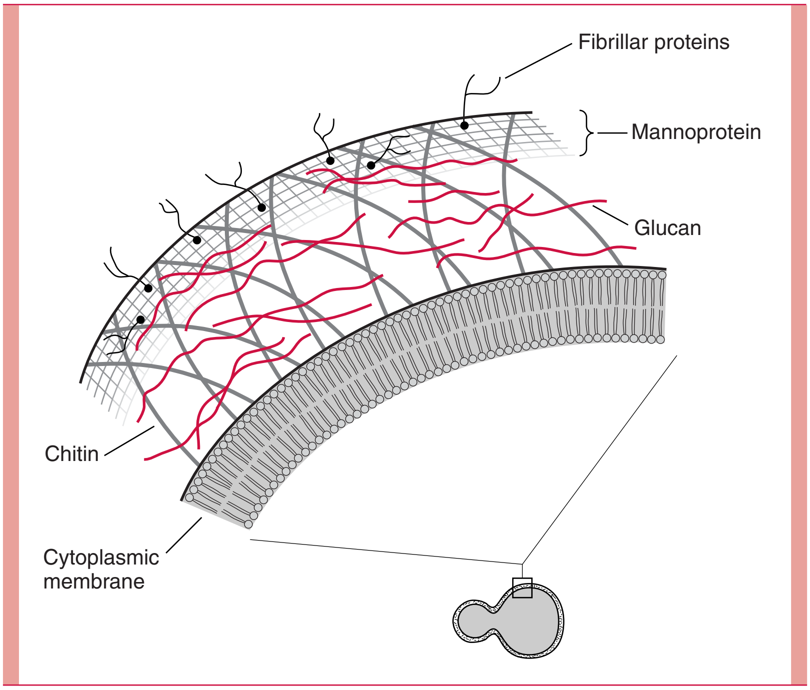


FIGURE 45–1
The fungal cell wall. The overlapping mannan, glucan, chitin, and protein elements are shown. Proteins complexed with the mannan (mannoproteins) extend beyond the cell wall

Cell wall mannan linked to surface proteins

Chitin and glucans give rigidity to cell wall

Mannoproteins are mannose-based polymers (mannan) found on the surface and in the structural matrix of the cell wall, where they are linked to protein. They are major determinants of serologic specificity because of variations in the composition and linkages of the polymer side chains. Glucans are glucosyl polymers, some of which form fibrils that increase the strength of the fungal cell wall, often in close association with chitin. Chitin is composed of long, unbranched chains of poly-*N*-acetylglucosamine. It is inert, insoluble, and rigid and provides structural support in a manner analogous to the chitin in crab shells or cellulose in plants. It is a major component of the cell wall of filamentous fungi. In yeasts, chitin appears to be of most importance in forming cross-septa and the channels through which nuclei pass from mother to daughter cells during cell division.

## METABOLISM

Heterotrophic metabolism uses available organic matter

Photosynthetic mechanisms are lacking

Fungal metabolism is heterotrophic, requiring exogenous carbon for growth. Metabolic diversity is great, but most fungi grow with only an organic carbon source and ammonium or nitrate ions as a nitrogen source. In nature, nutrients for free-living fungi are derived from decaying organic matter. A major difference between fungi and plants is that fungi lack photosynthetic energy-producing mechanisms. Most are strict aerobes, although some can grow under anaerobic conditions. None are strict anaerobes.

## REPRODUCTION

Asexual reproduction forms conidia by mitosis

Meiosis forms sexual spores in specialized structures

Fungi may reproduce by either asexual or sexual processes. Reproductive elements produced asexually are termed **conidia.** Those produced sexually are termed **spores** (e.g., ascospores, zygospores, basidiospores). Asexual reproduction involves mitotic division of the haploid nucleus and is associated with production by budding spore-like conidia or separation of hyphal elements. In sexual reproduction, the haploid nuclei of donor and recipient cells fuse to form a diploid nucleus, which may then divide by classical meiosis. Some of the four resulting haploid nuclei may be genetic recombinants and may undergo further division by mitosis. Highly complex specialized structures may be involved. Detailed study of this process in fungal species such as *Neurospora crassa* has been important in gaining an understanding of basic cellular genetic mechanisms.

## FUNGAL MORPHOLOGY AND GROWTH

The size of fungi varies immensely. A single cell without transverse septa may range from bacterial size (2–4 $\mu$m) to a macroscopically visible structure. The morphologic forms of growth vary from colonies superficially resembling those of bacteria to some of the most complex, multicellular, colorful, and beautiful structures seen in nature. Mushrooms are an example and can be regarded as a complex organization of cells showing structural differentiation.

Vary from bacterial size to multicellular mushrooms

**Mycology,** the science devoted to the study of fungi, has many terms to describe the morphologic components that make up these structures. Fortunately, the terms and concepts that must be mastered can be limited by considering only the fungi of medical importance and accepting some simplification.

### YEASTS AND MOLDS

Initial growth from a single cell may follow either of two courses, yeast or mold (Fig 45–2). The first and simplest is the formation of a bud, which extends out from a

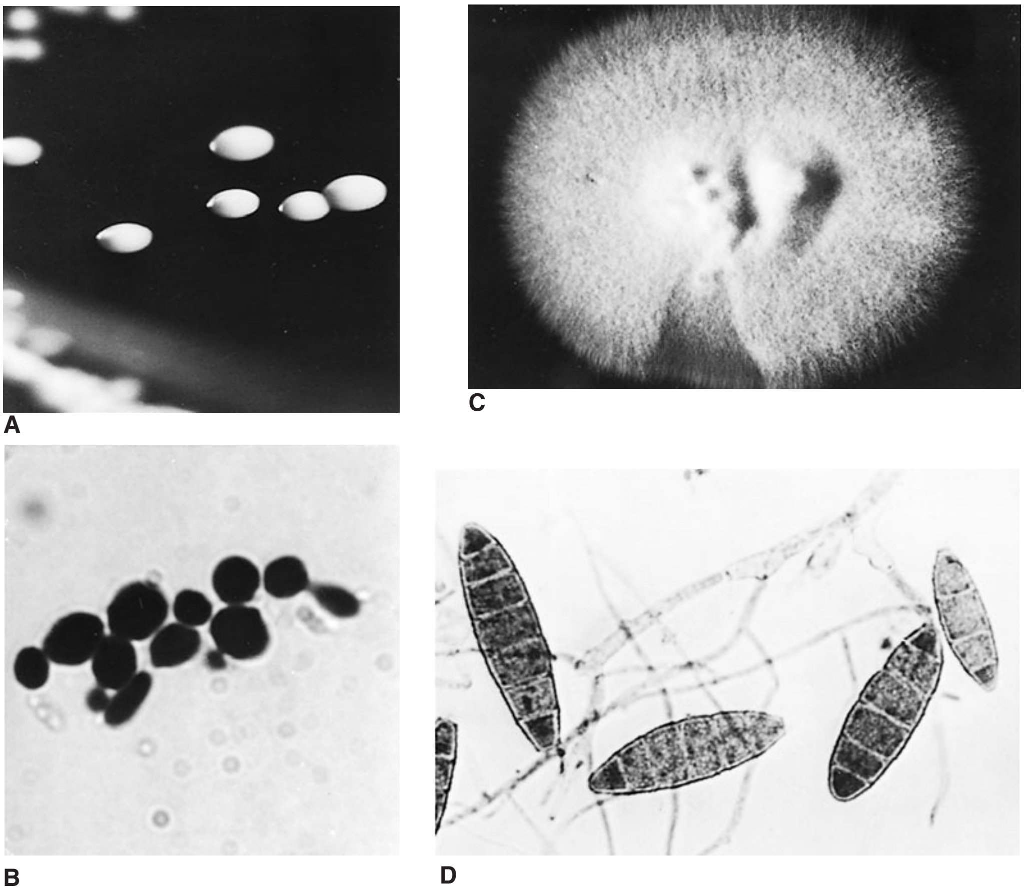


FIGURE 45–2
Yeast and mold forms of fungal growth. **A.** Yeasts form colonies similar to those of bacteria. **B.** Microscopically, they are large oval cells with occasional buds (blastoconidia). **C.** Molds form fuzzy, often pigmented colonies. **D.** Microscopically, molds are a complex of hyphae and associated conidia. (*Parts* C *and* D *reprinted with permission from Dr. E. S. Beneke and the Upjohn Company: Scope Publications, Human Mycoses.*)

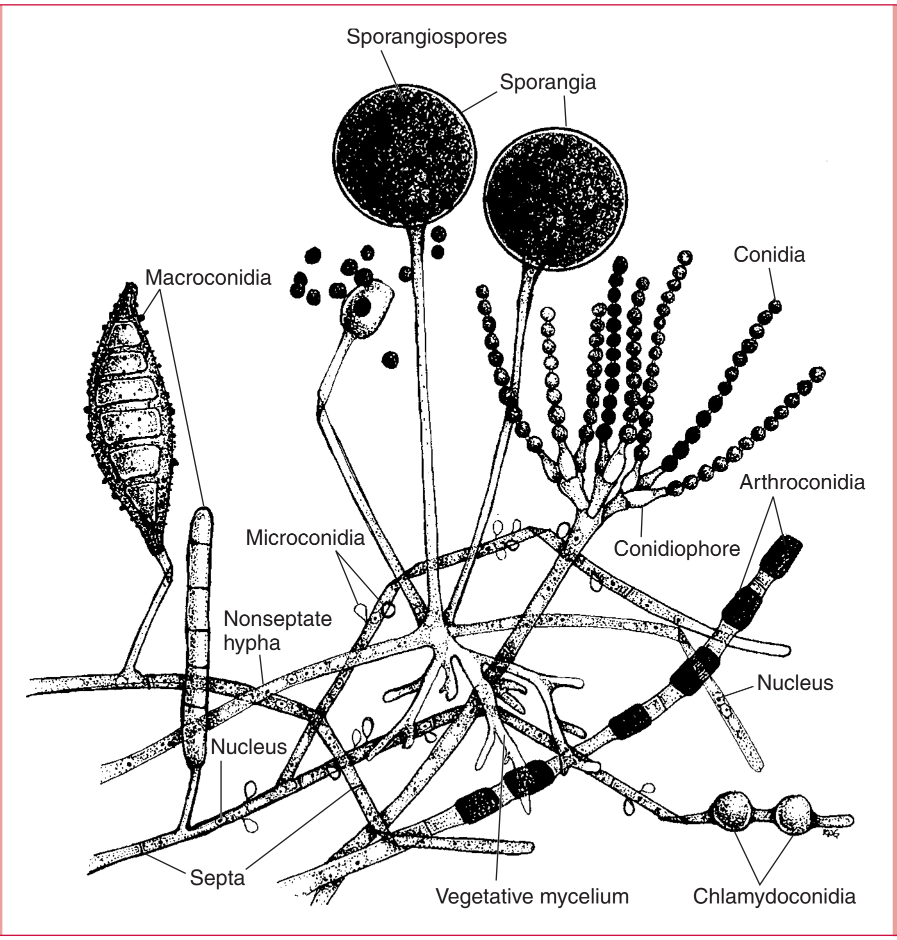


FIGURE 45–3
Mold forms. The tube-like hyphae constitute their basic structure. Examples of spores and conidia and of the structures that bear them are shown. They develop from the hyphal wall.

Yeasts produce blastoconidia by budding

round or oblong parent, constricts, and forms a new cell. These buds are called **blastoconidia** (see Fig 45–2), and fungi that reproduce in this manner are called **yeasts.** On plates, yeasts form colonies that resemble those of bacteria. In broth, yeasts produce diffuse turbidity or grow as sediments in unshaken cultures.

Molds produce septate or nonseptate hyphae

Vegetative mycelium acts as a root

Aerial mycelium bears reproductive conidia or spores

Pseudohyphae are less rigid

Fungi may also grow through the development of **hyphae** (singular, hypha), which are tube-like extensions of the cell with thick, parallel walls. As the hyphae extend, they form an intertwined mass called a **mycelium.** Most fungi form hyphal **septa** (singular, septum), which are cross-walls perpendicular to the cell walls that divide the hypha into subunits (Fig 45–3). These septa may not form complete walls and vary among species in the extent to which they restrict movement of organelles and nuclei. Some species are nonseptate; they form hyphae and mycelia as a single, continuous cell. In both septate and nonseptate hyphae, multiple nuclei are present, with free flow of cytoplasm along the hyphae or through pores in any septum. A portion of the mycelium (vegetative mycelium) usually grows into the medium or organic substrate (eg, soil) and functions like the roots of plants as a collector of nutrients and moisture. The more visible surface growth may assume a fluffy character as the mycelium becomes aerial. The hyphal walls are rigid enough to support this extensive, intertwining network, commonly called a **mold.** The aerial hyphae bear the reproductive structures of this class of fungi. Some fungi form structures called **pseudohyphae** (Fig 45–4), which differ from true hyphae in having recurring bud-like constrictions and less rigid cell walls.

Morphology of reproductive conidia and spores used for identification

The reproductive conidia and spores of the molds and the structures that bear them assume a great variety of sizes, shapes, and relationships to the parent hyphae, and the morphology and development of these structures are the primary basis of identification of medically important molds. The mycelial structure plays some role in identification,

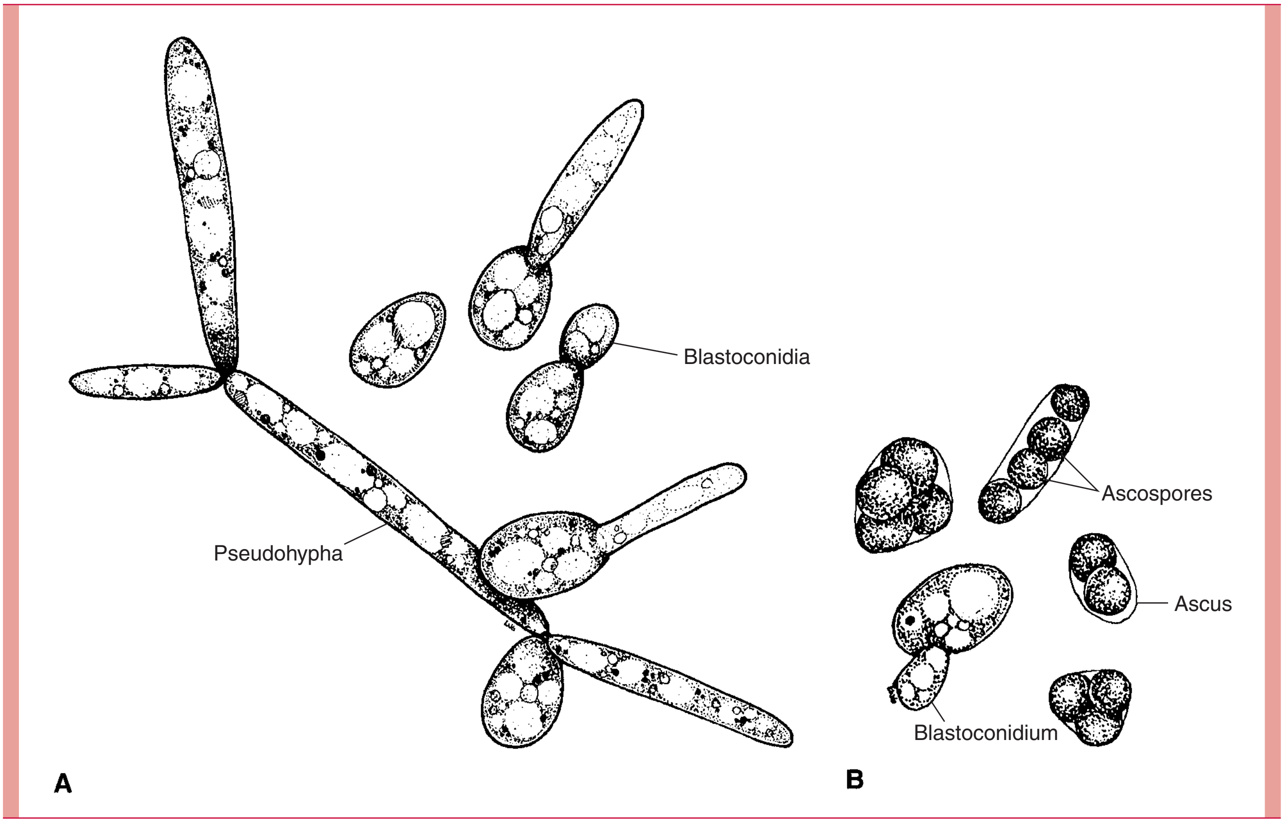


FIGURE 45–4
Yeast forms. **A.** Yeast reproduction is limited to the development of blastoconidia or longer extensions, pseudohyphae. **B.** Sexual reproduction leads to the formation of ascospores.

depending on whether the hyphae are septate or nonseptate, but differences are not sufficiently distinctive to identify or even suggest a fungal genus or species.

Exogenously formed conidia may arise directly from the hyphae or on a special stalk-like structure, the **conidiophore.** Occasionally, terms such as **macroconidia** and **microconidia** are used to indicate the size and complexity of these conidia. Conidia that develop within the hyphae are called either **chlamydoconidia** or **arthroconidia.** Chlamydoconidia become larger than the hypha itself; they are round, thick-walled structures that may be borne on the terminal end of the hypha or along its course. Arthroconidia conform more to the shape and size of the hyphal units but are thickened or otherwise differentiated. Arthroconidia may form a series of delicately attached conidia that break off and disseminate when disturbed. The most common sexual spore is termed an **ascospore.** Four or eight ascospores may be found in a sac-like structure, the **ascus.** The structures are illustrated in Figures 45–3 and 45–4.

Conidia and conidiophore arrangements determine names

Ascospores are borne in ascus sac

## DIMORPHISM

In general, fungi grow either as yeasts or as molds; mold forms show the greatest diversity. Some species can grow in either a yeast or a mold phase, depending on environmental conditions. These species are known as **dimorphic fungi.** Several human pathogens demonstrate dimorphism; they grow in the mold form in their environmental reservoir and in culture at ambient temperatures, but convert to the yeast or some other form in infected tissue. For most, it is possible to manipulate the cultural conditions to demonstrate both yeast and mold phases in vitro. Yeast phase growth requires conditions similar to those of the parasitic in vivo environment, such as 35 to 37°C incubation and enriched

Dimorphic fungi grow as yeasts in tissue or molds in environment

Infectious conidia disseminate molds

medium. Mold growth requires minimal nutrients and ambient temperatures. The conidia produced in the mold phase may be infectious and serve to disseminate the fungus.

## CLASSIFICATION

Taxonomy is based on sexual spores and septation of hyphae

Asexual form is unknown for most pathogens

rRNA genes are used for classification

Although conidia are more readily observed, the major classification of fungi primarily depends on the nature of sexual spores and septation of hyphae as its differential characteristics. On this basis, fungi have been organized into four to six classes or phyla. A major problem of classifying the medically important fungi using these groups is that for most species, no sexual form has been demonstrated. This may be due to its loss during evolution or because the spores are so rarely produced that they have not been detected. One approach has been to give these fungi their own class (Deuteromycetes, or **fungi imperfecti**) and wait for the discovery of the sexual form to place it in one of the legitimate groups—the Ascomycetes, Basidiomycetes, or Zygomycetes. The application of molecular methods such as analysis of ribosomal RNA genes has allowed the placement of species pending discovery of the sexual forms. The medically important genera are shown in Table 45–1. Discovery of the sexual form may not bring immediate clarity from the student's standpoint; for instance, when the sexual stage of *Trichophyton mentagrophytes* was demonstrated, it was found to be identical to that of an already named ascomycete (*Arthroderma benhamiae*). Most medically important species are now assigned to the Ascomycetes and a few to the Basidiomycetes or Zygomycetes.

Medical grouping organized by biological behavior in humans

Systemic fungi infect previously healthy persons

The grouping of medically important fungi used in the following chapters is based on the types of tissues they parasitize and the diseases they produce, rather than on the principles of basic mycologic taxonomy. The **superficial** fungi, such as the dermatophytes, cause indolent lesions of the skin and its appendages, commonly known as ringworm and athlete's foot. The **subcutaneous** pathogens characteristically cause infection through the skin, followed by subcutaneous spread, lymphatic spread, or both. The **opportunistic** fungi are those found in the environment or in the normal flora that occasionally produce disease, usually in the compromised host. The **systemic** pathogens are the most virulent fungi and may cause serious progressive systemic disease in previously healthy persons. They are not members of the normal human flora. Although their major potential is to produce deep-seated visceral infections and systemic spread (systemic mycoses), they

TABLE 45–1

**Classification of Medically Important Fungi**

| Genus | Typical Growth | Septation[a] | Sexual Form | Phylum | Medical Classification |
|---|---|---|---|---|---|
| *Aspergillus* | Mold | + | ? | Ascomycete | Opportunistic |
| *Blastomyces* | Dimorphic | + | ? | Ascomycete | Systemic |
| *Candida* | Dimorphic | + | ? | Ascomycete | Opportunistic |
| *Coccidioides* | Dimorphic | + | ? | Ascomycete | Systemic |
| *Cryptococcus* | Yeast | | + | Basidiomycete | Systemic |
| *Epidermophyton* | Mold | + | + | Ascomycete | Superficial |
| *Histoplasma* | Dimorphic | + | + | Ascomycete | Systemic |
| *Microsporum* | Mold | + | + | Ascomycete | Superficial |
| *Mucor* | Mold | − | + | Zygomycete | Opportunistic |
| *Pneumocystis* | Cysts[b] | | ? | Ascomycete | Opportunistic |
| *Rhizopus* | Mold | − | + | Zygomycete | Opportunistic |
| *Sporothrix* | Dimorphic | + | ? | Ascomycete | Subcutaneous |
| *Trichophyton* | Mold | + | + | Ascomycete | Superficial |

[a] For those that form hyphae.

[b] Tissue forms but does not grow in culture.

may also produce superficial infections as part of their disease spectrum or as the initiating event. The superficial mycoses do not spread to deeper tissues. As with all clinical classifications, overlaps and exceptions occur. In the end, the organism defines the disease, and it must be isolated or otherwise demonstrated.

## EPIDEMIOLOGY

Most fungal infections arise from contact with an environmental reservoir or from the patient's own fungal flora. Some superficial mycoses can be transmitted from person to person by very close contact, such as sharing a comb with an individual who has scalp ringworm; others can be acquired from ringworm infections of animals. Other fungal infections are not communicable between humans or animals, and infected patients need not be isolated.

Infection is from environment or endogenous flora

Only dermatophyte infections are communicable

## DIAGNOSIS

Because of their large size, fungi often demonstrate distinctive morphologic features on direct microscopic examination of infected pus, fluids, or tissues. The simplest method is to mix the specimen with a 10% solution of potassium hydroxide (KOH) preparation and place it under a coverslip. The strong alkali digests or clears the tissue elements (epithelial cells, leukocytes, debris) but not the rigid cell walls of both yeasts and molds. After digestion of the material, the fungi can be observed under the light microscope with or without staining (see Fig 47–1B). Some yeasts stain with common stains such as the Gram stain, to which they are usually positive. Direct examinations can be aided by the use of calcifluor white, a dye that binds to polysaccharides in cellulose and chitin. Under ultraviolet light, calcifluor white fluoresces, enhancing detection of fungi in fluids or tissue sections.

KOH digests tissue but not fungal wall

Some yeasts are Gram-positive

Calcifluor white enhances detection

Histopathologic examination of tissue biopsy specimens is widely used and shows the relationship of the organism to tissue elements and responses (blood vessels, phagocytes, granulomatous reactions). Most fungi can be seen in sections stained with the hematoxylin and eosin (H&E) method routinely used in histology laboratories (Fig 45–5). Specialized staining procedures such as the silver impregnation methods are frequently used because they stain almost all fungi strongly but only a few tissue components. The pathologist should be alerted to the suspicion of fungal infection when tissues are submitted, because special stains and searches for fungi are not made routinely.

Often visible in H&E preparations

Silver stains enhance detection

Fungi can be grown by methods similar to those used to isolate bacteria. Growth occurs readily on enriched bacteriologic media commonly used in clinical laboratories (eg, blood agar and chocolate agar). Many fungal cultures, however, require days to weeks of incubation for initial growth; bacteria present in the specimen grow more rapidly and may interfere with isolation of a slow-growing fungus. Therefore, the culture procedures of diagnostic mycology are designed to favor the growth of fungi over bacteria and to allow incubation to continue for a sufficient time to isolate slow-growing strains.

Growth in culture is simple but slow

Selective media allow isolation in the presence of bacteria

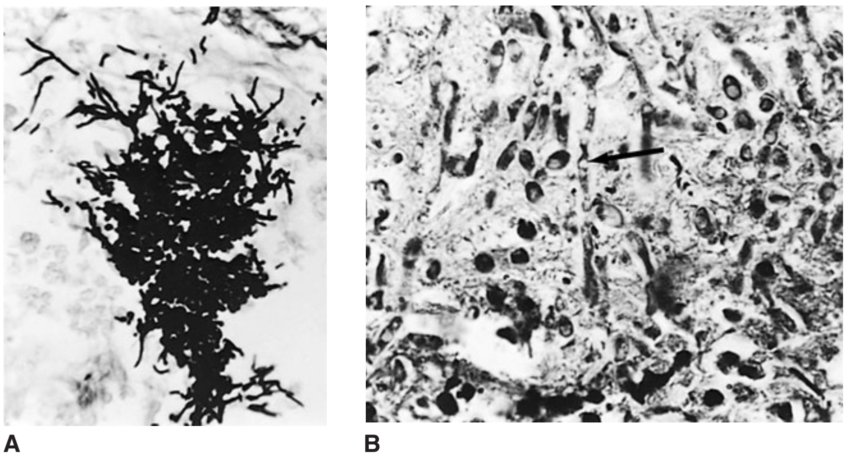


FIGURE 45–5 Direct examinations for fungi. **A.** Fungi such as *Candida albicans* are large enough to be demonstrated microscopically at low magnification. **B.** In histologic sections the invasive pseudohyphae (arrow) may be seen. (*Part* A *reproduced with permission from Dr. E. S. Beneke and the Upjohn Company: Scope Publications, Human Mycoses.*)

Sabouraud's agar optimal for fungi but poor for bacteria

The most commonly used medium for cultivating fungi is Sabouraud's agar, which contains only glucose and peptones as nutrients. Its pH is 5.6, which is optimal for growth of dermatophytes and satisfactory for growth of other fungi. Most bacteria associated with humans fail to grow or grow poorly on Sabouraud's agar.

Selective media make use of antimicrobics

Cultures incubated at 30°C for primary isolation

Blood agar or another enriched bacteriologic agar medium is used when pure cultures would be expected. It is made selective for fungi by the addition of antibacterial antibiotics such as chloramphenicol and gentamicin. Cycloheximide, an antimicrobic that inhibits some saprophytic fungi, is sometimes added to Sabouraud's agar to prevent overgrowth of contaminating molds from the environment, particularly for skin cultures. Media containing these selective agents cannot be relied on exclusively because they can interfere with growth of some pathogenic fungi or because the "contaminant" may be producing an opportunistic infection. For example, cycloheximide inhibits *Cryptococcus neoformans,* and chloramphenicol may inhibit the yeast forms of some dimorphic fungi. Selective media are not needed for growing fungi from sterile sites such as cerebrospinal fluid or tissue biopsy specimens. In contrast to most parasitic bacteria, many fungi grow best at 25 to 30°C, and temperatures in this range are used for primary isolation. Paired cultures incubated at 30 and 35°C may be used to demonstrate dimorphism.

Yeast identified biochemically

Once a fungus is isolated, identification procedures depend on whether it is a yeast or mold. Yeasts are identified by biochemical tests analogous to those used for bacteria, including some that are identical (eg, urease production). The ability to form pseudohyphae is also taxonomically useful among the yeasts.

Molds identified by morphology and culture features

Molds are most often identified by the morphology of their conidia and conidiophores. Other features such as the size, texture, and color of the colonies help characterize molds, but without demonstrating conidiation they are not sufficient for identification. The ease and speed with which various fungi produce conidia vary greatly. Minimal nutrition, moisture, good aeration, and ambient temperature favor development of conidia.

Lactophenol cotton blue stains mycelia, conidia, and spores

Microscopic fungal morphology is usually demonstrated by methods that allow in situ microscopic observation of the fragile asexual conidia and their shape and arrangement. Morphology may also be examined in fragments of growth teased free of a mold and examined moist in preparations containing a dye called lactophenol cotton blue. The dye stains the hyphae, conidia, and spores. Conidium production may not occur for days or weeks after the initial growth of the mold. It is somewhat like waiting for flowers to bloom, and it can be frustrating when the result has immediate clinical application.

Temperature variation demonstrates dimorphism

Exoantigen and DNA probes are more rapid

It is desirable, but not always possible, to demonstrate both the yeast and mold phases with dimorphic fungi. In some cases, this result can be achieved with parallel cultures at 30° and 35°C. The tissue form of *Coccidioides immitis* is not readily produced in vitro. An alternate approach has been developed for identification of some of the dimorphic systemic fungi, based on soluble antigens prepared from mycelial growth (exoantigens) and called the exoantigen test. When these exoantigens react with specific antibody in an immunodiffusion procedure, precipitin lines are formed between the unknown antigen and its homologous antibody. Results are usually available much more rapidly than are results of cultural tests. For a few fungi, DNA probes are available for rapid speciation.

Serologic tests are useful for systemic fungi

Serum antibodies directed against a variety of fungal antigens can be detected in patients infected with those agents. Except for some of the systemic pathogens, the sensitivity, specificity, or both, of these tests have not been sufficient to recommend them for use in diagnosis or therapeutic monitoring of fungal infections. The tests of value are discussed in sections on specific agents.

## ADDITIONAL READING

Ajello L, Hay RJ (eds). Medical Mycology. Volume 4 in Collier L, Balows A, Sussman, M (eds). *Topley & Wilson's Microbiology and Microbial Infection*. London: Arnold, 1998. This classic British reference work now devotes an entire volume to fungi and fungal diseases beautifully written and illustrated by experts from both sides of the Atlantic.

Guarro J, Gené J, Stchigel AM. Developments in fungal taxonomy. *Clin Microbiol Rev* 1999;12:454–500. This review is a preview of what is ahead with the application of molecular methods to fungal taxonomy.

CHAPTER 46

# Pathogenesis, Immunity, and Chemotherapy of Fungal Infections

KENNETH J. RYAN

We all have regular contact with fungi. They are so widely distributed in our environment that thousands of fungal spores are inhaled or ingested every day. Other species are so well adapted to humans that they are common members of the normal flora. Despite this ubiquity, clinically apparent systemic fungal infections are quite uncommon, even among persons living within the geographic habitat of the more pathogenic species. However, progressive systemic fungal infections pose some of the most difficult diagnostic and therapeutic problems in infectious disease, particularly among immunocompromised patients to whom they are a major threat. The purpose of this chapter is to give an overview of the pathogenesis and immunology of fungal infections and of the activity of antifungal agents. Details relating to specific fungi are given in Chapters 47 to 50.

## GENERAL ASPECTS OF FUNGAL DISEASE

### EPIDEMIOLOGY

Fungal infections are acquired from the environment or may be endogenous in the few instances where they are members of the normal flora. Inhalation of infectious conidia generated from molds growing in the environment is a common mechanism. Some of these molds are ubiquitous, whereas others are restricted to geographic areas whose climate favors their growth. In the latter case, disease can be acquired only in the endemic area. Some environmental fungi produce disease after they are accidentally injected past the skin barrier. The pathogenic fungi represent only a tiny fraction of those found in the environment. Endogenous infections are restricted to a few yeasts, primarily *Candida albicans*. These yeasts have the ability to colonize by adhering to host cells and, given the opportunity, invade deeper structures.

Environmental conidia are inhaled or injected

Endogenous yeasts may invade

## PATHOGENESIS

Fungal pathogenesis is similar to bacteria

Most fungi are opportunists

Compared with bacterial, viral, and parasitic disease, less is known about the pathogenic mechanisms and virulence factors involved in fungal infections. Analogies with bacterial diseases come the closest because of the apparent importance of adherence to mucosal surfaces, invasiveness, extracellular products, and interaction with phagocytes. In general, the principles discussed in Chapter 10 apply to fungal infections. Most fungi are opportunists, producing serious disease only in individuals with impaired host defense systems. Only a few fungi are able to cause disease in previously healthy persons.

### Adherence

Adherence is mediated by fungal adhesins and host cell receptors

Mannoprotein is an adhesin, and fibronectin a receptor

A number of fungal species, particularly the yeasts, are able to colonize the mucosal surfaces of the gastrointestinal and female genital tracts. It has been shown experimentally that the ability to adhere to buccal or vaginal epithelial cells is associated with colonization and virulence. Within the genus *Candida* (see Chapter 48), the species that adhere best to epithelial cells are those most frequently isolated from clinical infections. Adherence usually requires a surface adhesin on the microbe and a receptor on the epithelial cell. In the case of *C. albicans,* mannoprotein components extending from the cell wall have been implicated as the adhesin and fibronectin, and other components of the extracellular matrix as the receptor(s). A few binding mediators have been identified for other fungi, usually a surface mannoprotein.

### Invasion

Traumatic injection is linked to trauma

Small conidia may pass airway defenses

Passing an initial surface barrier, whether skin, mucous membrane, or respiratory epithelium, is an important step for most successful pathogens. Some fungi are introduced through mechanical breaks. For example, *Sporothrix schenckii* infection typically follows a thorn prick or some other obvious trauma (see Chapter 47). Fungi that initially infect the lung must produce conidia small enough to be inhaled past the upper airway defenses. For example, arthroconidia of *Coccidioides immitis* (2 to 6 $\mu$m) can remain suspended in air for a considerable time and can reach the terminal bronchioles to initiate pulmonary coccidioidomycosis.

Invasion across mucosal barriers may involve enzymes

Triggered by temperature and possibly other cues, dimorphic fungi from the environment undergo a metabolic shift similar to the heat shock response and completely change their morphology and growth to a more invasive form. Invasion directly across mucosal barriers by the endogenous yeast *C. albicans* is similarly associated with a morphologic change, the formation of hyphae. The triggering mechanisms of this change are unknown, but the new form is able to penetrate and spread. Extracellular enzymes (eg, proteases, elastases) are associated with the hyphal form of *Candida* and with the invasive forms of many of the dimorphic and other pathogenic fungi. Although these enzymes must contribute to some aspect of invasion or spread, their precise role is unknown for any fungus.

### Tissue Injury

No classic exotoxins are produced in vivo

Injury is due to inflammatory and immunologic responses

None of the extracellular products of opportunistic fungi or dimorphic pathogens have been shown to injure the host directly during infection in a manner analogous to bacterial toxins. Although the presence of necrosis and infarction in the tissues of patients with invasion by fungi such as *Aspergillus* suggests a toxic effect, direct evidence is lacking. A number of fungi do produce exotoxins, called **mycotoxins,** in the environment but not in vivo. The structural components of the cell do not cause effects similar to those of the endotoxin of Gram-negative bacteria, although mannan is known to circulate widely in the body. The injury caused by fungal infections seems to be due primarily to the inflammatory and immune responses that are stimulated by the prolonged presence of the fungus.

## IMMUNITY

### Phagocyte Interactions

There is considerable evidence that normal persons have a high level of natural resistance to most fungal infections. This is particularly true of opportunistic molds. An important component of this resistance is the ability of healthy neutrophils to kill hyphae of most fungi if they reach the tissues. A small number of species, all of which are dimorphic, are able to produce mild to severe disease in otherwise healthy individuals. In vitro studies have shown these fungi to be more resistant to killing by neutrophils than the opportunists. *C. albicans* is able to bind complement components in a way that interferes with phagocytosis.

Most fungi are readily killed by neutrophils

*C. immitis,* one of the best-studied species, has been shown to contain a component in the wall of its conidial (infective) phase that is antiphagocytic. As the hyphae convert to the spherule (tissue) phase, they also become resistant to phagocytic killing because of their size and surface characteristics. The tissue yeast form of *Histoplasma capsulatum* is resistant to phagocytic killing after ingestion and, in fact, multiplies within macrophages. These mechanisms of avoiding phagocytic killing appear to allow many dimorphic fungi to multiply sufficiently to produce an infection that can be controlled only by the immune response.

Tissue phases of dimorphic fungi resist phagocytic killing

### Adaptive Immune Response

A recurrent theme with fungal infections is the importance of an intact immune response in preventing infection and progression of disease. Most fungi are incapable of producing even a mild infection in immunocompetent individuals.

A small number of species are able to cause clinically apparent infection that usually resolves once there is time for activation of normal immune responses. In most instances in which it has been investigated, the actions of neutrophils and T lymphocyte–mediated immune responses have been found to be of primary importance in this resolution. Progressive, debilitating, or life-threatening disease with these agents is commonly associated with depressed or absent cell-mediated immune responses, and the course of any fungal disease is worse in immunocompromised than previously healthy persons.

T cell–mediated responses of primary importance

Progressive fungal diseases occur in the immunocompromised

### *Humoral Immunity*

Antibodies can be detected at some time during the course of almost all fungal infections, but for most there is little evidence that they contribute to immunity. The only encapsulated fungus, *Cryptococcus neoformans,* is one example of a fungus against which antibody plays a role in controlling infection. Although the polysaccharide capsule of *C. neoformans* has antiphagocytic properties similar to those of encapsulated bacterial pathogens (eg, *Streptococcus pneumoniae, Haemophilus influenzae*), it is less antigenic. Anticapsular antibody plays a role in resolving cryptococcal infection, but T cell–mediated responses are still dominant. Antibody also plays a role in control of *C. albicans* infections by enhancing fungus–phagocyte interactions, and this is probably true for other yeasts. In some other fungal infections, the lack of protective effect of antibody is striking. In coccidioidomycosis, for example, high titers of *C. immitis*–specific antibodies are associated with dissemination and a worsening clinical course.

Opsonizing antibody is effective in some yeast infections

### *Cellular Immunity*

Considerable clinical and experimental evidence points toward the importance of cellular immunity in fungal infections. Most patients with severe systemic disease have neutropenia, defects in neutrophil function, or depressed T lymphocyte–mediated immune reactions. These can result from factors such as steroid treatment, leukemia, Hodgkin's disease, and acquired immunodeficiency syndrome. In other cases, an immunologic deficit can usually be demonstrated by absence of delayed-type hypersensitivity responses or by direct in vitro assays of T-cell responsiveness to the fungus in question. In

Systemic disease associated with deficiencies in neutrophils and T cell–mediated immunity

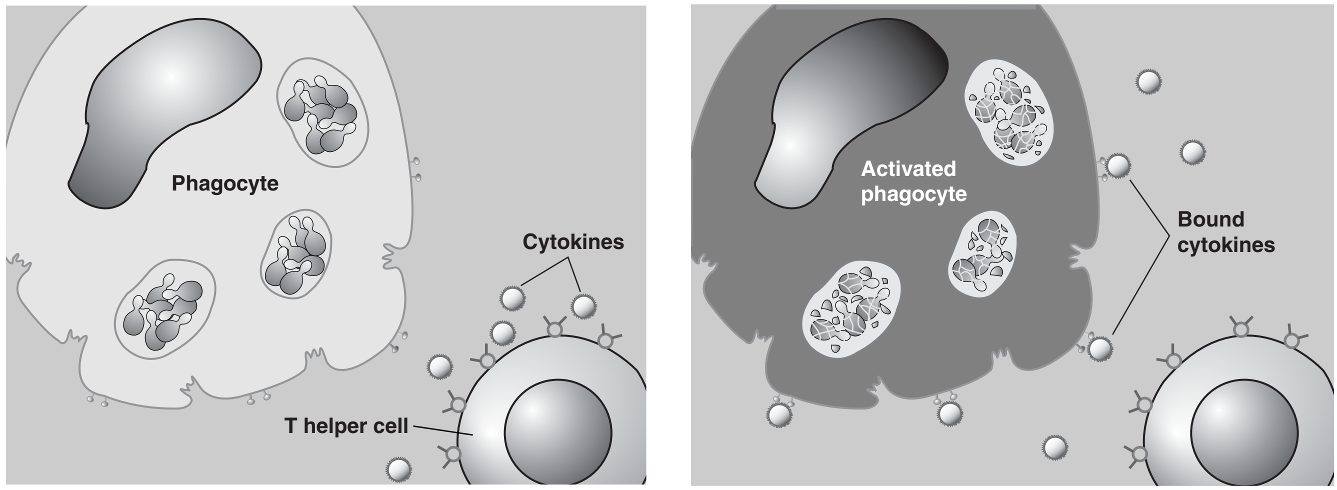


FIGURE 46–1
Cell-mediated immunity to fungal infections. **A.** Most pathogenic fungi are able to survive and multiply in phagocytes (**B**) until the phagocyte becomes specifically activated by cytokines from T cells. The growth is then restricted with development of cell-mediated immunity.

the latter case, it is possible that hyporesponsiveness is due at least in part to activation of suppressor cells or continued circulation of fungal antigen.

Fungi that escape neutrophils grow slowly in macrophages

Growth is restricted when macrophages activated by cytokines

Immune defects lead to progressive disease

Although not all fungi have been studied to the same degree, a unified picture emerging from clinical and experimental animal studies is illustrated in Figure 46–1. When hyphae or yeast cells of the fungus reach deep tissue sites, they are either killed by neutrophils or resist destruction by one of the antiphagocytic mechanisms described earlier. Surviving cells continue to grow slowly or, if they are dimorphic, convert to their yeast, hyphal, or spherule tissue phases. The growth of these invasive forms may be slowed but not killed by macrophages. In healthy persons, the extent of infection is small, and any symptoms are caused by the inflammatory response. Everything awaits the specific immune response. The turning point comes when the macrophages are activated by cytokine mediators produced by T lymphocytes that have interacted with the fungal antigen. Where they have been identified, these mediators, which are associated with helper T-cell responses, are those such as interleukin 2 or interferon-$\gamma$. The activated macrophages are then able to restrict the growth of the fungus, and the infection is controlled. Defects that disturb this cycle lead to progressive disease. To the extent that they are known, the specifics of these reactions are discussed in the following chapters.

## ANTIFUNGAL CHEMOTHERAPY

Many antifungals are too toxic for use

Compared with antibacterial agents, relatively few antimicrobics are available for treatment of fungal infections. Many substances with antifungal activity have proved either to be unstable, to be toxic to humans, or to have undesirable pharmacologic characteristics, such as poor diffusion into tissues. Of the agents in current clinical use, none approaches the degree of selective toxicity that $\beta$-lactams provide for antibacterial therapy, but the newer azole compounds have significantly higher therapeutic activity and lower toxicity than earlier antifungal agents.

Fortunately, most fungal infections are self-limiting and require no chemotherapy. Superficial mycoses are often treated, but topical therapy can be used, thus limiting toxicity to the host. The remaining small group of deep mycoses that are uncontrolled by the host's immune system require the prolonged use of relatively toxic antifungals. This, combined with the fact that most of the patients have underlying immunosuppression, makes them the most difficult of all infectious diseases to treat successfully. The characteristics of currently used antifungal agents are discussed next and summarized in Table 46–1.

Treatment is most needed for dissemination in immunocompromised persons

## ANTIFUNGAL ANTIMICROBICS THAT AFFECT MEMBRANE STEROLS

### Polyenes

The polyenes **nystatin** and **amphotericin B** are lipophilic and bind to sterols in the cytoplasmic membrane of eukaryotic cells. Following binding, they form annular channels, which penetrate the membrane and lead to leakage of essential small molecules from the cytoplasm and cell death. The basis of their selective toxicity is their greater affinity for the sterols of fungal membranes, such as ergosterol, than the sterols of human cells. This difference is relative, because they also bind cholesterol in mammalian membranes, which creates the considerable toxicity that limits their use.

Sterol binding forms membrane channels

At physiologic pH, amphotericin B is insoluble in water and must be administered intravenously as a colloidal suspension. Amphotericin B is not absorbed from the gastrointestinal tract. Almost all fungi are susceptible to amphotericin B, and the development of resistance is too rare to be a consideration in its use. The major limitation to amphotericin B therapy is the toxicity created by its affinity for mammalian as well as fungal membranes. Infusion is commonly followed by chills, fever, headache, and dyspnea. The most serious toxic effect is renal dysfunction and is seen in virtually every patient receiving a therapeutic course. Experienced clinicians learn to titrate the dosage for each patient to minimize the nephrotoxic effects. For obvious reasons, use of amphotericin B is limited to progressive, life-threatening fungal infections. In these cases, despite its toxicity, it often remains the antifungal agent of choice. Preparations that complex amphotericin B with phospholipids to form liposomes have been used as a way to limit toxicity. The even greater toxicity of nystatin limits its use to topical preparations.

Insoluble compound must be infused in suspension

Active against most fungi

Therapy must be titrated against toxicity

### Azoles

The azoles are a large family of synthetic organic compounds, which includes members with antibacterial, antifungal, and antiparasitic properties. The important antifungal azoles are the imidazole, **ketoconazole,** and the triazoles, **fluconazole** and **itraconazole.** Others are under development or evaluation. Their activity is based on inhibition of a cytochrome enzyme (P450 demethylase) responsible for conversion of lanosterol to ergosterol, the major component of the fungal cytoplasmic membrane. This leads to lanosterol accumulation and the formation of a defective cell membrane with altered permeability characteristics.

Enzyme inhibition is crucial for ergosterol synthesis

Ketoconazole was the first azole to be useful in systemic infections but is now being supplanted by either fluconazole or itraconazole for most systemic mycoses, including aspergillosis and candidiasis, for which ketoconazole was not effective. Ketoconazole and itraconazole are given orally, and fluconazole, either orally or intravenously. Although nausea, vomiting, and elevation of hepatic enzymes complicate the treatment of some patients, the azoles are much less toxic than amphotericin B. Endocrinologic defects can be a problem because of inhibition of conversion of lanosterol to cholesterol, a precursor of several hormones. Central nervous system penetration of ketoconazole is poor, which limits its effectiveness in systemic coccidioidomycosis and cryptococcosis, but fluconazole has been more effective. Currently, fluconazole and itraconazole are the primary alternates to amphotericin B for treatment of systemic fungal infections. Azoles are also effective for superficial and subcutaneous mycoses in which the initial therapy either fails or is not tolerated by the patient. Two other azoles, **clotrimazole** and **miconazole,** are used in over-the-counter topical preparations.

All are less toxic than amphotericin B

Fluconazole, itraconazole are most used

**Voriconazole,** a second-generation azole, inhibits both 14-$\alpha$-sterol demethylase and 24-methylene dihydrolanosterol demethylation, providing a broader spectrum of activity

Voriconazole has broad-spectrum activity

TABLE 46–1

## Features of Antifungal Agents

| Agent | Mechanism of Action | Mechanism of Resistance | Route | Clinical Use |
|---|---|---|---|---|
| **Polyenes** | | | | |
| Nystatin | Membrane disruption | Sterol modification | Topical | Most fungi |
| Amphotericin B | Membrane disruption | Sterol modification | Intravenous | Most fungi |
| **Azoles** | | | | |
| Ketoconazole | Demethylase block of ergosterol synthesis | Active efflux, demethylase alteration, or overproduction[a] | Oral | *Candida, Cryptococcus,* dimorphic fungi[b] |
| Fluconazole | Demethylase block of ergosterol synthesis | Active efflux, demethylase alteration, or overproduction[a] | Oral, intravenous | *Candida, Cryptococcus,* dimorphic fungi |
| Itraconazole | Demethylase block of ergosterol synthesis | Active efflux, demethylase alteration, or overproduction[a] | Oral, intravenous | *Candida, Cryptococcus,* dimorphic fungi, invasive molds (*Aspergillus*) |
| Clotrimazole | Demethylase block of ergosterol synthesis | Unknown[c] | Topical | *Candida,* some other yeasts |
| Miconazole | Demethylase block of ergosterol synthesis | Unknown[c] | Topical | *Candida,* some other yeasts |
| Voriconazole | Demethylase block of ergosterol synthesis | Unknown[c] | Oral, intravenous | *Candida,* some other yeasts and molds |
| **Allylamines** | | | | |
| Terbinafine | Squalene accumulation | ?Active efflux | Oral | Dermatophytes, combined with azoles for *Candida, Aspergillus* |
| Naftifine | Squalene accumulation | Unknown | Topical | Dermatophytes |
| **Flucytosine** | | | | |
| | RNA and DNA synthesis | Permease or modifying enzymes[d] absent or decreased | Oral | *Candida* and *Cryptococcus,* resistance emerges in monotherapy |
| **Echinocandins** | | | | |
| Caspofungin | Block of glucan synthesis | Unknown | Intravenous | *Aspergillus, Candida* |
| **Griseofulvin** | Microtubule disruption | Unknown | Oral | Dermatophytes |
| **Potassium iodide** | Unknown | Unknown | Oral | *Sporothrix schenckii* |
| **Tolnaftate** | Unknown | Unknown | Oral | Dermatophytes |

*Abbreviation:* 5FC, 5-flucytosine.

[a] Most work is with fluconazole and *Candida,* other azoles are to be assumed similar.

[b] Generally less absorbed and less active than fluconazole or itraconazole.

[c] Probably similar to other azoles, but resistance to the concentrations in topical preparations may differ.

[d] Cytosine deaminase and uracil phosphoribosyltransferase (the enzyme that forms 5-fluorodoxyuridine from 5FC).

against some yeasts and molds that are resistant to the other azoles. It can be given intravenously or orally.

## Allylamines

The allylamines are a group of synthetic compounds that act by inhibition of an enzyme (squalene epoxidase) in the early stages of ergosterol synthesis. Their lethal effect is due to accumulation of squalene precursors rather than a deficiency of ergosterol. The allylamines include an oral agent, **terbinafine,** and a topical agent, **naftifine.** Both are used in the treatment of dermatophyte (ringworm) infections.

Cause squalene accumulation

# ANTIFUNGALS THAT AFFECT NUCLEIC ACID SYNTHESIS

## Flucytosine

**5-Flucytosine** (5FC), which was originally developed as an anticancer drug, is an antimetabolite analog of cytosine. It is a potent inhibitor of RNA, DNA, and ultimately protein synthesis. 5FC enters the cell aided by a permease, where it is converted to 5-fluorouracil by the action of cytosine deaminase. After further modification, 5FC is incorporated into what becomes defective RNA. Its effect on DNA synthesis is through its conversion to another metabolite (5-fluorodeoxyuridine), which is a potent inhibitor of thymidylate synthetase.

Enzymatically modified form makes defective RNA

Inhibits DNA synthesis

Flucytosine is well absorbed after oral administration. It is active against most clinically important yeasts, including *C. albicans* and *C. neoformans,* but has little activity against molds or dimorphic fungi. A significant limitation is the development of resistance that can occur by single-step mutation during therapy. Potential resistance limits flucytosine use to mild yeast infections or treatment in combination with amphotericin B for life-threatening systemic infections. Use in combination reduces the chance for expression of flucytosine resistance and allows a lower dose of amphotericin B to be used. In some instances, the combination is synergistic. The primary toxic effect of flucytosine is a reversible bone marrow suppression that can lead to neutropenia and thrombocytopenia. This effect is dose related and can be controlled by drug monitoring.

Active against yeasts but not molds

Resistance develops during therapy if used alone

# ANTIFUNGALS THAT AFFECT CELL WALL SYNTHESIS

Given the unique chemical nature of the fungal cell wall, with its interwoven layers of mannan, mannoprotein, glucan, and chitin (see Chapter 45), it is disappointing that agents that act on any component of the cell wall have not yet made an impact in antifungal chemotherapy. The fungal equivalent of the $\beta$-lactam and glycopeptide inhibitors of bacterial peptidoglycan synthesis would be most welcome. One class of agents (echinocandins), which block glucan synthesis by inhibition of glucan synthetase, cause morphologic distortions and osmotic instability in yeast that are similar to the effect of $\beta$-lactams on bacteria. The first such agent to be licensed is **caspofungin,** which has good activity against *Candida* and *Aspergillus*. Another class of compounds (nikomycins), which disrupt chitin synthesis, are at an earlier stage of development.

Agents acting on glucan and chitin synthesis are emerging

# OTHER ANTIFUNGAL AGENTS

**Griseofulvin** is a product of a species of the mold *Penicillium*. It is active only against the agents of superficial mycoses. Griseofulvin is actively taken up by susceptible fungi and acts on the microtubules and associated proteins that make up the mitotic spindle. It interferes with cell division and possibly other cell functions associated with microtubules. Griseofulvin is absorbed from the gastrointestinal tract after oral administration and concentrates in the keratinized layers of the skin. Clinical effectiveness has been demonstrated for all causes of dermatophyte infection, but the response is slow. Difficult cases may require 6 months of therapy to effect a cure.

Microtubule disruption interferes with cell division

Active against dermatophytes

**Potassium iodide** is the oldest known oral chemotherapeutic agent for a fungal infection. It is effective only for cutaneous sporotrichosis. Its activity is somewhat paradoxical, because the mold form of the etiologic agent, *Sporothrix schenckii,* can grow on medium containing 10% potassium iodide. The pathogenic yeast form of this dimorphic fungus appears to be susceptible to molecular iodine. **Tolnaftate** is a derivative of naphthiomate. It has activity against dermatophytes (see Chapter 47) but not against yeasts. It has been effective in topical treatment of dermatophytoses and is available in over-the-counter preparations.

Iodide inhibits *Sporothrix*

## RESISTANCE TO ANTIFUNGAL AGENTS

### Definition of Resistance

The concepts, definitions, and laboratory methods described in Chapter 14 for bacterial resistance are generally applicable to fungi. Quantitative susceptibility is measured by the minimal inhibitory concentration (MIC) under conditions that favor the growth of fungi. The diversity of growth rates and metabolic activity in the various fungi has made application of the MIC to therapy more difficult than in bacteria. The MICs performed in different types of fungal growth media can vary as much as 1000-fold. Although which medium is "right" cannot be determined, there has now been agreement on a standardized broth dilution method so experimental and clinical results can be reliably compared. Most of this work is with yeasts; molds are more difficult to work with and not suited to testing in broth. As with bacteria, fungi with MICs in the pharmacologically achievable range may or may not be clinically susceptible. Because of the variables cited above, high MICs do not predict resistance with the same certainty they do with bacteria. For these reasons, antifungal susceptibility testing is still considered investigational and not offered in hospital laboratories.

Concepts are similar to bacterial resistance

Laboratory methods are variable

### Mechanisms of Resistance

The cell wall and cytoplasmic membrane present a barrier for antifungal agents to access the fungal interior. While this is generally considered a mechanism of innate resistance, there have been examples in which changes in membrane sterols appear to have restricted permeability to azoles. 5FC requires entry of a permease into the cell, and the absence of this enzyme is a significant mechanism of acquired resistance. Energy-requiring efflux pumps, which remove the drug from the cytoplasm, appear to be an even more important mechanism of resistance with the azoles. The efflux mechanism may confer resistance to multiple agents, and the mechanisms and genes involved are similar to the human P-glycoprotein pump associated with resistance to antineoplastic chemotherapeutics.

Alteration in membrane sterols restricts access

Efflux pumps remove drug from cytoplasm

Alterations in the target of the antifungal agent are an important means of acquired resistance. Although resistance to polyenes is rare, it has been traced to the appearance in the cytoplasmic membrane of sterols that have a decreased affinity for these agents. The production of cytochrome demethylases with lower affinity for azoles is also associated with resistance. Other mechanisms of resistance involve the absence or overproduction of crucial enzymes. Isolates resistant to 5FC lack either the permease or the cytosine deaminase that converts it to its active form. Resistance to both azoles and allylamines has been associated with overproduction of their target enzymes. It is surprising that enzymatic inactivation, the most potent bacterial resistance mechanism, is not important for any of the antifungals in current use.

Altered targets include membrane and enzymes

Overproduction of target enzymes negates effect

Inactivating enzymes are not produced

## SELECTION OF ANTIFUNGALS

As with all chemotherapy, the selection of antifungal agents for treatment of superficial, subcutaneous, and systemic mycoses involves balancing probable efficacy against toxicity. The factors to be considered are (1) the threat of morbidity or mortality posed by the specific infection, (2) the immune status of the patient, (3) the toxicity of the antifungal, and (4) the probable activity of the antifungal agent against the fungus. In the case of superficial mycoses, the risks of appropriate therapy are small, and a number of topical agents may be tried. At the other extreme, an immunocompromised patient will most

Immune status dictates aggressiveness of therapy

Azoles with or without amphotericin B are the standard likely be treated aggressively with systemic agents for proven or even suspected systemic fungal infection. Amphotericin B, despite its toxicity, is still the treatment of choice for almost all serious systemic fungal infections, but the newer azoles are often added. The most common regimen is an initial course of amphotericin B followed by one of the azoles.

## ADDITIONAL READING

Calderone RA, Cihlar RL (eds). *Fungal Pathogenesis: Principles and Clinical Applications.* New York: Marcel Dekker; 2002. This volume is a collection of papers that gives the current status of advances in understanding of the pathogenesis and immune mechanisms of the major systemic and opportunistic pathogens.

Ghannoum MA, Rice LB. Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *Clin Microbiol Rev* 1999;12:501–517. This review focuses on mechanisms and includes interesting discussions of parallels with bacterial resistance (where they exist).

Kontoyiannis DP, Lewis RE. Antifungal drug resistance of pathogenic fungi. *Lancet* 2002;359:1135–1144. This concise review covers mechanisms of action and resistance and includes comprehensive tables on both topics.

Rex JH, Pfaller MA, Walsh TJ, et al. Antifungal susceptibility testing: Practical aspects and current challenges. *Clin Microbiol Rev* 2001;14:643–658. This review centers around the application of the method adopted as a standard in the United States but also discusses future methods (flow cytometry, biochemical tests) and the difficulty of testing molds.

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CHAPTER 47

# Dermatophytes, *Sporothrix*, and Other Superficial and Subcutaneous Fungi

KENNETH J. RYAN

The least invasive of fungi are the dermatophytes and other superficial fungi that are adapted to the keratinized outer layers of the skin. The subcutaneous fungi go a step further extending to the tissue beneath the skin but rarely invade deeper. Both are discussed here and summarized in Table 47–1.

## SUPERFICIAL FUNGI

## Dermatophytes

Dermatophytoses are superficial infections of the skin and its appendages, commonly known as ringworm, athlete's foot, and jock itch. They are caused by species of the genera *Microsporum, Trichophyton,* and *Epidermophyton,* which are collectively known as dermatophytes. These fungi are highly adapted to the nonliving, keratinized tissues of nails, hair, and the stratum corneum of the skin. The source of infection may be humans, animals, or the soil.

### MYCOLOGY

Dermatophytes (literally, skin-plants) are molds that have been classified as Deuteromycetes (fungi imperfecti). The three genera of medical importance are *Epidermophyton, Microsporum,* and *Trichophyton,* which are separated primarily by the morphology of their macroconidia and presence of microconidia. The sexual forms have been discovered for many of the *Microsporum* and *Trichophyton* species and assigned to ascomycete

Form septate hyphae, macroconidia, and microconidia

TABLE 47–1

**Agents of Superficial and Subcutaneous Mycoses**

| Fungus | Fungal Growth: In Lesion | Fungal Growth: In Culture (25°C) | Infection Site | Disease |
|---|---|---|---|---|
| **Dermatophytes** | | | | |
| *Microsporum canis* | Septate hyphae | Mold | Hair,[a] skin | Ringworm |
| *Microsporum audouini* | Septate hyphae | Mold | Hair[a] | Ringworm |
| *Microsporum gypseum* | Septate hyphae | Mold | Hair, skin | Ringworm |
| *Trichophyton tonsurans* | Septate hyphae | Mold | Hair, skin, nails | Ringworm |
| *Trichophyton rubrum* | Septate hyphae | Mold | Hair, skin, nails | Ringworm |
| *Trichophyton mentagrophytes* | Septate hyphae | Mold | Hair, skin | Ringworm |
| *Trichophyton violaceum* | Septate hyphae | Mold | Hair, skin, nails | Ringworm |
| *Epidermophyton floccosum* | Septate hyphae | Mold | Skin | Ringworm |
| **Other superficial fungi** | | | | |
| *Malassezia furfur*[b] | Yeast (mycelia)[c] | Yeast | Skin (pink to brown)[d] | Pityriasis (tinea) versicolor |
| *Hortaea werneckii*[e] | Septate hyphae, ellipsoidal cells | Yeast (mold) | Skin (brown–black)[d] | Tinea nigra |
| *Trichosporon cutaneum* | Septate hyphae | Mold | Hair (white)[b] | White piedra |
| *Piedraia hortae* | Septate hyphae | Mold, ascospores | Hair (black)[b] | Black piedra |
| **Subcutaneous fungi** | | | | |
| *Sporothrix schenckii* | Cigar-shaped yeast (rare) | Mold | Subcutaneous, lymphatic spread | Sporotrichosis |
| *Fonsecaea pedrosoi* | Muriform body[f] | Mold | Wart-like foot lesions | Chromoblastomycosis |
| *Phialophora verrucosa* | Muriform body[f] | Mold | Wart-like foot lesions | Chromoblastomycosis |
| *Cladophialophora (Cladosporium) carrionii* | Muriform body[f] | Mold | Wart-like foot lesions | Chromoblastomycosis |

[a] Specimens fluoresce under ultraviolet light.
[b] Previously known as *Pityrosporum orbiculare*.
[c] Denotes less frequent findings.
[d] Color of clinical lesions.
[e] Previously known as *Cladosporium werneckii.*
[f] Multicompartment yeast-like structure.

*Epidermophyton, Microsporum,* and *Trichophyton* are major genera

Grow best at 25°C

genera (*Arthroderma, Nannizzia*). Dermatophytes are still called by their previous names in the medical literature for reasons of familiarity and because identification procedures continue to be based on the characteristics of their conidia. Many species cause dermatophyte infections; the most common of these are shown in Table 47–1. They require a few days to a week or more to initiate growth. Most grow best at 25°C on Sabouraud's agar, which is usually used for culture. The hyphae are septate, and their conidia may be borne directly on the hyphae or on conidiophores. Small microconidia may or may not be formed; however, the larger and more distinctive macroconidia (Fig 47–1C) are usually the basis for identification.

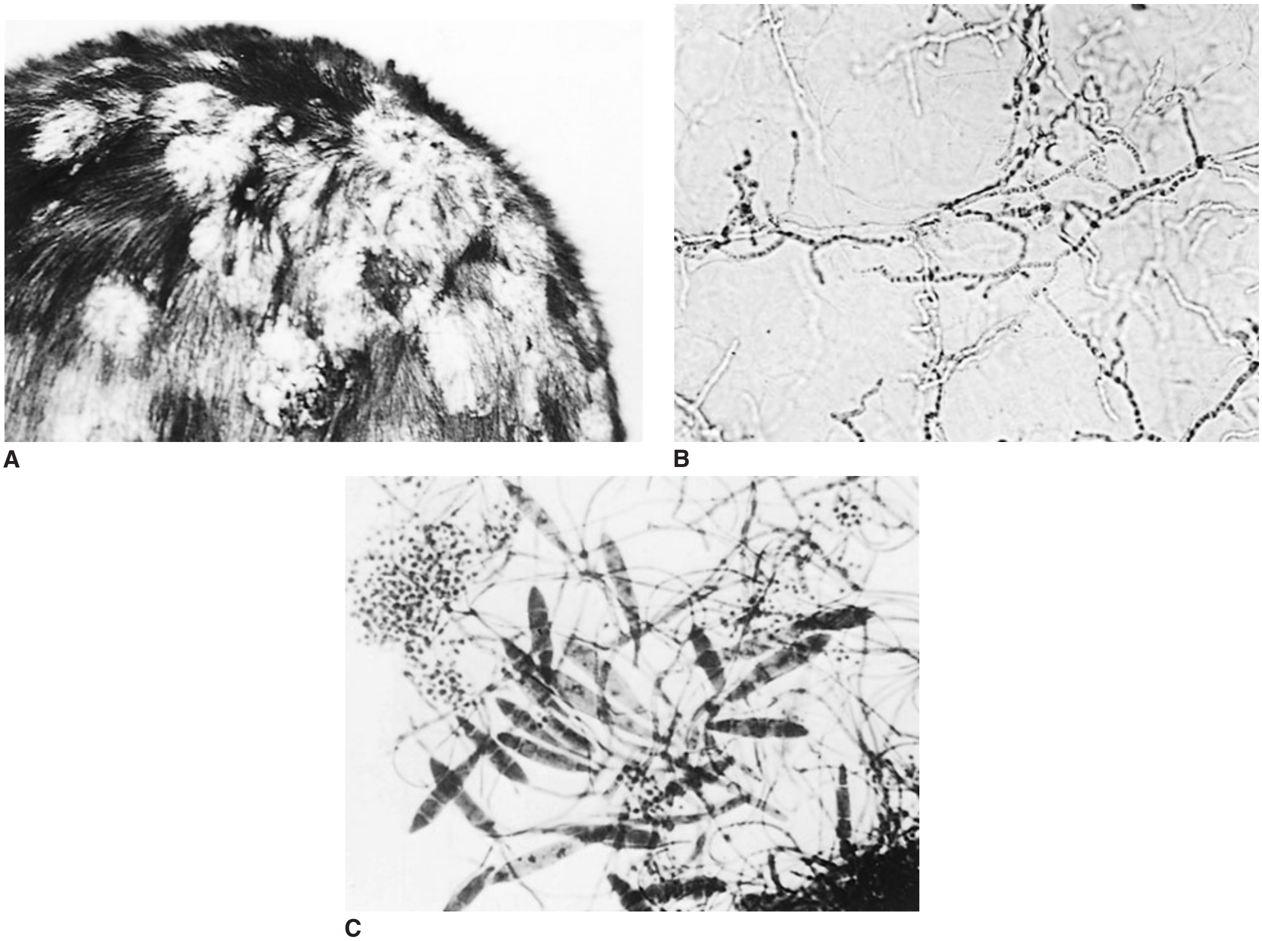

FIGURE 47–1

Dermatophyte infection of scalp (ringworm). **A.** Scalp lesions. Note the annular margination. **B.** Scrapings taken from the edge of the scalp lesion in KOH. Only the hyphal elements are visible. **C.** Culture. Hyphae, macroconidia, and microconidia are present. The macroconidia are characteristic of *Trichophyton*. (*Reprinted with permission from Dr. E. S. Beneke and the Upjohn Company: Scope Publications, Human Mycoses.*)

## DERMATOPHYTE DISEASE

CLINICAL CAPSULE

Dermatophytoses are slowly progressive eruptions of the skin and its appendages which may be unsightly but are not painful or life threatening. The manifestations (and names) vary depending on the nature of the inflammatory response in the skin, but typically involve erythema, induration, itching, and scaling. The most familiar is "ringworm," which gets its name from the annular shape of creeping margin at the advancing edge of dermatophyte growth.

### EPIDEMIOLOGY

Reservoir may be human, animal, or soil

There are both ecologic and geographic differences in the occurrence of the various dermatophyte species. Some are primarily adapted to the skin of humans, others to animals, and others to the environment. All may serve as the source for human infection. Many wild and domestic animals, including dogs and cats, are infected with certain dermatophyte species and represent a large reservoir for infection of humans. There are large differences between temperate and tropical climates in the frequency of cases and isolations from nonhuman sources of the different species. Many of these differences are changing with shifts in population.

Transmission requires contact with intact or detached skin or hair

Human-to-human transmission usually requires close contact with an infected subject or infected person or animal, because dermatophytes are of low infectivity and virulence. Transmission usually takes place within families or in situations involving contact with detached skin or hair, such as barber shops and locker rooms. No special precautions beyond handwashing need be taken by the medical attendant after contact with an infected patient.

## PATHOGENESIS

Initial infection is through minor skin breaks

Balance between fungal growth and skin desquamation determines outcome

Dermatophytoses begin when minor traumatic skin lesions come in contact with dermatophyte hyphae shed from another infection. Susceptibility may be enhanced by local factors such as the composition surface fatty acids. Once the stratum corneum is penetrated, the organism can proliferate in the keratinized layers of the skin aided by a variety of proteinases. The course of the infection is dependent on the anatomic location, moisture, the dynamics of skin growth and desquamation, the speed and extent of the inflammatory response, and the infecting species. For example, if the organisms grow very slowly in the stratum corneum, and turnover by desquamation of this layer is not retarded, the infection will probably be short-lived and cause minimal signs and symptoms. Inflammation tends to increase skin growth and desquamation rates and helps limit infection, whereas immunosuppressive agents such as corticosteroids decrease shedding of the keratinized layers and tend to prolong infection. Invasion of any deeper structures is extremely rare.

Poor inflammatory response leads to chronic infection

Most infections are self-limiting, but those in which fungal growth rates and desquamation are balanced and in which the inflammatory response is poor tend to become chronic. The lateral spread of infection and its associated inflammation produce the characteristic sharp advancing margins that were once believed to be the burrows of worms. This characteristic is the origin of the common name **ringworm** and the Latin term **tinea** (worm) that is often applied to the clinical forms of the disease (Fig 47–1A).

Hair shaft is penetrated and broken by hyphae

Infection may spread from skin to other keratinized structures, such as hair and nails, or may invade them primarily. The hair shaft is penetrated by hyphae, which extend as arthroconidia either exclusively within the shaft (endothrix) or both within and outside the shaft (ectothrix). The end result is damage to the hair shaft structure, which often breaks off. Loss of hair at the root and plugging of the hair follicle with fungal elements may result. Invasion of the nail bed causes a hyperkeratotic reaction, which dislodges or distorts the nail.

## IMMUNITY

Delayed hypersensitivity responses occur

CMI responses are the most important

The great majority of dermatophyte infections pass through an inflammatory stage to spontaneous healing. Phagocytes are able to use oxidative pathways to kill the fungi both intracellularly and extracellularly. Little is known about the factors that mediate the host response in these self-limiting infections or whether they confer immunity to subsequent exposures. Antibodies may be formed during infection but play no known role in immunity. Most clinical and experimental evidence points to the importance of cell-mediated immunity (CMI), as with other fungal infections. The timing of the inflammatory response to infection correlates with appearance of delayed hypersensitivity, and resolution of infection is associated with the blastogenic T-lymphocyte responses. Enhanced desquamation with the inflammatory response helps remove infected skin.

Widespread infection is associated with T-lymphocyte defects and *T. rubrum*

Occasionally, dermatophyte infections become chronic and widespread. This progression has been related to both host and organism factors. Approximately half of these patients have underlying diseases affecting their immune responses or are receiving treatments that compromise T-lymphocyte function. These chronic infections are particularly associated with *Trichophyton rubrum,* to which both normal and immunocompromised persons appear to be hyporesponsive. Although a number of mechanisms have been proposed, how this organism is able to grow without stimulating much inflammation is unexplained.

# DERMATOPHYTOSES: CLINICAL ASPECTS

## MANIFESTATIONS

Dermatophyte infections range from inapparent colonization to chronic progressive eruptions that last months or years, causing considerable discomfort and disfiguration. Dermatologists often give each infection its own "disease" name, for example, tinea capitis (scalp), tinea pedis (feet, athlete's foot), tinea manuum (hands), tinea cruris (groin), tinea barbae (beard, hair), and tinea unguium (nail beds). Skin infections not included in this anatomic list are called tinea corporis (body). There are some general clinical, etiologic, and epidemiologic differences between these syndromes, but there is also considerable overlap. The primary differences between etiologic agents that infect different sites are shown in Table 47–1.

Various skin sites are labeled as tinea "diseases"

Infection of hair begins with an erythematous papule around the hair shaft, which progresses to scaling of the scalp, discoloration, and eventually fracture of the shaft. Spread to adjacent hair follicles progresses in a ring-like fashion, leaving behind broken, discolored hairs and sometimes black dots where the hair is absent but the infection has gone into the follicle. The degree of inflammatory response markedly affects the clinical appearance and, in some cases, can cause constitutional symptoms. In most cases, symptoms beyond itching are minimal.

Hair infection leads to itching and hair loss

Skin lesions begin in a similar pattern and enlarge to form sharply delineated erythematous borders with skin of nearly normal appearance in the center. Multiple lesions can fuse to form unusual geometric patterns on the skin. Lesions may appear in any location, but are particularly common in moist, sweaty skin folds. Obesity and the wearing of tight apparel increase susceptibility to infection in the groin and beneath the breasts. Another form of infection, which involves scaling and splitting of the skin between the toes, is commonly known as athlete's foot. Moisture and maceration of the skin provide the mode of entry.

Skin infection favors moist areas and skin folds

Nail bed infections first cause discoloration of the subungual tissue, then hyperkeratosis and apparent discoloration of the nail plate by the underlying infection follow. Direct infection of the nail plate is uncommon. Progression of hyperkeratosis and associated inflammation cause disfigurement of the nail but few symptoms until the nail plate is so dislodged or distorted that it exposes or compresses adjacent soft tissue.

Hyperkeratosis can dislodge the nail bed

## DIAGNOSIS

The goal of diagnostic procedures is to distinguish dermatophytoses from other causes of skin inflammation. Infections caused by bacteria, other fungi, and noninfectious disorders (psoriasis, contact dermatitis) may have similar features. The most important step is microscopic examination of material taken from lesions to detect the fungus. Potassium hydroxide (KOH) or calcifluor white preparations of scales scraped from the advancing edge of a dermatophyte lesion demonstrate septate hyphae (Fig 47–1B). Examination of infected hairs reveals hyphae and arthroconidia penetrating the hair shaft. Broken hairs give the best yield. Some species of dermatophyte fluoresce, and selection of hairs for examination can be aided by the use of an ultraviolet lamp (Wood's lamp).

KOH mounts of skin scrapings and infected hairs demonstrate hyphae

Some species fluoresce

The same material used for direct examination can be cultured for isolation of the offending dermatophyte. Mild infections with typical clinical findings and positive KOH preparations are often not cultured, because clinical management is not influenced significantly by the identity of the etiologic species. Clinically typical infections with negative KOH preparations require culture. The major reason for false-negative KOH results, however, is failure to collect the scrapings or hairs properly.

Culture is used when KOH preparations negative

## TREATMENT AND PREVENTION

Many local skin infections resolve spontaneously without chemotherapy. Those that do not may be treated with topical tolnaftate, allylamines, or azoles. Nail bed and more extensive

Topical tolnaftate, allylamines, or azoles usually sufficient

Systemic griseofulvin, or azoles used in refractory cases

skin infections require systemic therapy with griseofulvin or itraconazole and terbinafine, often combined with topical therapy. Therapy must be continued over weeks to months, and relapses may occur. Keratolytic agents may be useful for reducing the size of hyperkeratotic lesions. Dermatophyte infections can usually be prevented simply by observing general hygienic measures. No specific preventive measures such as vaccines exist.

## *Other Superficial Mycoses*

M. furfur requires lipids for growth

**Pityriasis (tinea) versicolor** occurs in tropical and temperate climates; it is characterized by discrete areas of hypopigmentation or hyperpigmentation associated with induration and scaling. Lesions are found on the trunk and arms; some assume pigments ranging from pink to yellow-brown, hence the term **versicolor.** Members of the genus *Malassezia,* of which *M. furfur* is the most common, are the cause; these organisms can be seen in skin scrapings as clusters of budding yeast cells mixed with hyphae. They grow in the yeast form in culture media enriched with lipids.

*H. werneckii* causes black lesions

**Tinea nigra,** another tropical infection, is characterized by brown to black macular lesions, usually on the palms or soles. There is little inflammation or scaling, and the infection is confined to the stratum corneum. The cause, *Hortaea werneckii,* is a black-pigmented fungus found in soil and other environmental sites. Scrapings of the lesion show brown–black-pigmented septate hyphae. In culture initial growth is in the yeast form, with slow development of hyphal elements.

Black or white piedra are infections of hair shaft

**Piedra** is an infection of the hair characterized by black or white nodules attached to the hair shaft. White piedra (caused by *Trichosporon cutaneum*) infects the shaft in hyphal forms, which fragment with occasional buds. Black piedra (caused by *Piedraia hortae*) shows branched hyphae and ascospores in sections of the hair.

## SUBCUTANEOUS FUNGI

Assignment of fungal organisms to the category of subcutaneous fungi is somewhat arbitrary, because fungal pathogens can produce many subcutaneous manifestations as part of their disease spectrum. Those considered here are introduced traumatically through the skin and involve mainly subcutaneous tissues, lymphatic vessels, and contiguous tissues. They rarely spread to distant organs. The diseases they cause include sporotrichosis, chromoblastomycosis, and mycetoma. Only sporotrichosis has a single specific etiologic agent, *Sporothrix schenckii*. Chromoblastomycosis and mycetoma are clinical syndromes with multiple fungal etiologies.

## *Sporothrix*

### *Sporothrix schenckii*

Mold conidiophores convert to cigar-shaped yeast

*S. schenckii* is a dimorphic fungus that grows as a cigar-shaped, 3- to 5-mm yeast (Fig 47–2) in tissues and in culture at 37°C. In addition to the mannan, glucan, and chitin found in the cell wall of other fungi, the cell wall of *S. schenckii* contains a unique substance, L-rhamnose, in complexes with mannan. The mold, which grows in culture at 25°C, is presumably the infectious form in nature. The hyphae are thin and septate, producing clusters of conidia at the end of delicate conidiophores (see Fig 47–2B).

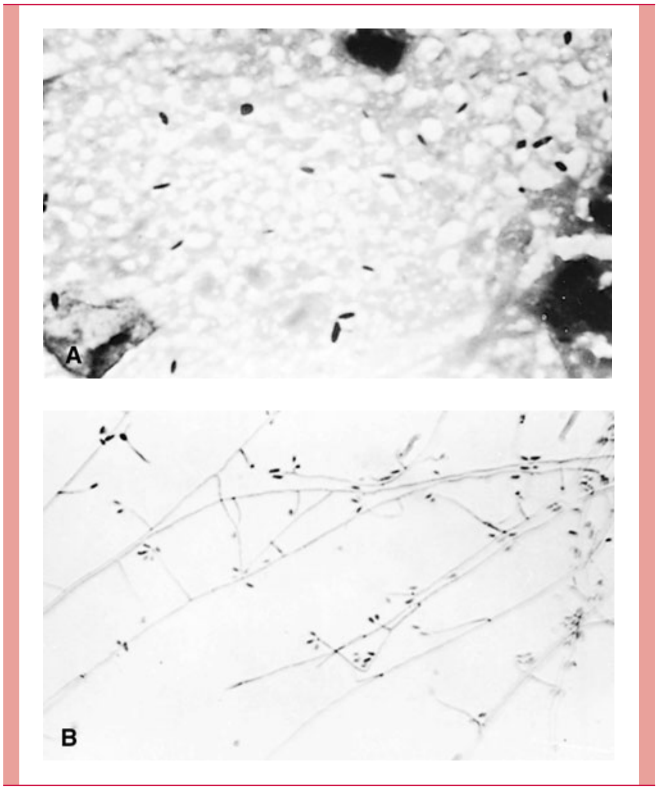

FIGURE 47–2
*Sporothrix schenckii*. **A.** The yeast form of *S. schenckii* is typically cigar-shaped but is rarely seen in human lesions. This smear is from infected mouse testis. **B.** Mold-phase cultures develop delicate hyphae and conidiophores bearing finger-like clusters of conidia. (*Reprinted with permission from Dr. E. S. Beneke and the Upjohn Company: Scope Publications, Human Mycoses.*)

# SPOROTRICHOSIS

## EPIDEMIOLOGY

*S. schenckii* is a ubiquitous saprophyte particularly found in hay, moss, soil, and decaying vegetation, and on the surfaces of various plants. Infection is acquired by traumatic inoculation through the skin of material containing the organism. Exposure is largely occupational or related to hobbies. The skin of gardeners, farmers, and rural laborers is frequently traumatized by thorns or other material that may be contaminated with conidia of *S. schenckii*. An unusual outbreak of sporotrichosis involving nearly 3000 miners was traced to *S. schenckii* in the timbers used to support mine shafts. A 1988 outbreak covered 15 states and was traced to sphagnum moss. Infection is occasionally acquired by direct contact with infected pus or through the respiratory tract; these modes of infection, however, are much less common than the cutaneous route.

Soil saprophyte is introduced by trauma

Occupational disease of gardeners and farmers

Outbreaks involve wood and moss

## PATHOGENESIS

Both the conidia and yeast cells of *S. schenckii* are able to bind to extracellular matrix proteins like fibronectin, laminin, and collagen. This may aid their survival in the early stages of infection. Local multiplication of the organism stimulates both acute pyogenic and granulomatous inflammatory reactions. Melanin production may provide resistance to oxidative killing. Proteinases similar to those seen in other fungal pathogens are present but no connection to virulence has been established. The infection spreads along lymphatic drainage routes and reproduces the original inflammatory lesions at intervals. The organisms are scanty in human lesions.

Surface binds to extracellular matrix

Melanin resists oxidative killing

## IMMUNITY

The cellular response to *S. schenckii* is mixed. The increased frequency and greater severity of disseminated disease in patients with T-cell defects points to CMI as the primary immune mechanism. Antibody plays no known role in immunity.

CMI is primary immune mechanism

# SPOROTRICHOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

Skin papule eventually ulcerates

Lymphatic involvement creates multiple lesions

A skin lesion begins as a painless papule that develops a few weeks to a few months after inoculation. Its location can usually be explained by occupational exposure; the hand is most often involved. The papule enlarges slowly and eventually ulcerates, leaving an open sore. Draining lymph channels are usually thickened, and pustular or firm nodular lesions may appear around the primary site of infection or at other sites along the lymphatic drainage route (Fig 47–3). Once ulcerated, lesions usually become chronic. Multiple ulcers often develop if the disease is untreated. Symptoms are those directly related to the local areas of infection. Constitutional signs and symptoms are unusual.

Deep infection is rare

Occasionally, spread occurs by other routes. The bones, eyes, lungs, and central nervous system are susceptible to progressive infection if the organisms reach these organs; such spread, however, occurs in less than 1% of all cases. Primary pulmonary sporotrichosis occurs but is also rare.

## DIAGNOSIS

Direct microscopic examination for *S. schenckii* is usually unrewarding because there are too few organisms to detect readily with potassium hydroxide preparations. Even specially stained biopsy samples and serial sections are usually negative, although the presence of a histopathologic structure, the asteroid body, is suggestive. This structure is composed of *S. schenckii* yeast cells surrounded by amorphous eosinophilic "rays." Definitive diagnosis depends on culture of infected pus or tissue. The organism grows within 2 to 5 days on all media commonly used in medical mycology. Identification requires demonstration of the typical conidia and of dimorphism.

## TREATMENT AND PREVENTION

Potassium iodide works for cutaneous fungi

Amphotericin or itraconazole required for progressive disease

Cutaneous sporotrichosis is effectively treated with a saturated solution potassium iodide (SSKI) administered orally. Systemic infections require the use of amphotericin B and/or azoles. Clinical experience with itraconazole has been excellent, and it may become the treatment of choice for all but cutaneous sporotrichosis. Eradication of the environmental reservoir of *S. schenckii* is not usually practical, although the mine outbreak mentioned previously was stopped by applying antifungal agents to the mine shaft timbers.

FIGURE 47–3
Sporotrichosis. This infection began on the finger and has started to spread up the arm, leaving satellite lesions behind. (*Reproduced with permission from Connor DH, Chandler FW, Schwartz DA, Manz HJ, Lack EE (eds).* Pathology of Infectious Disease, *Volume II. Stamford, CT: Appleton & Lange, 1997.*)

## *Chromoblastomycosis*

Chromoblastomycosis is primarily a tropical disease caused by multiple species of *Fonsecaea, Phialophora,* and *Cladophialophora* (*Cladosporium*). The disease occurs typically on the foot or leg. It appears as papules that develop into scaly, wartlike structures, usually under the feet. Fully developed lesions have been likened to the tips of a cauliflower. Extension is by satellite lesions; it is slow and painless and does not involve the lymphatic vessels. The organisms are found in the soil of endemic areas, and most infections occur in individuals who work barefoot.

Multiple species produce wart-like pigmented lesions in tropics

The outstanding mycologic feature is the presence of brown-pigmented, thick-walled, multiseptate, 5- to 12-mm globose structures called muriform bodies on histologic section. Branching septate hyphae may also be demonstrated in KOH preparations of scrapings. Cultures grow as dark molds, but may take weeks to appear and longer for demonstration of characteristic conidia. Surgery and antifungal therapy have been used in chromoblastomycosis, but results in advanced disease are disappointing. Flucytosine or itraconazole have been the antifungal agents most frequently used.

Brown pigmented bodies are seen in tissues

## *Mycetoma*

Mycetoma is a clinical term for an infection associated with trauma to the foot which causes inoculation of any of a dozen fungal species. Actinomycetes such as *Nocardia* may produce a similar disease. The usual clinical appearance is of massive induration with draining sinuses. Some of the fungi that cause mycetoma are geographically widespread; most cases, however, occur in the tropics, probably because the chronically damp, macerated skin of the feet that causes predisposition toward mycetoma occurs most often among those who go barefoot in the tropical environment. This finding is illustrated by the case of a college rower in Seattle who developed mycetoma; he was the only member of his shell who insisted on rowing barefoot. Once established, the treatment of mycetoma is difficult. No antimicrobic stands out as particularly helpful. The precise microbiologic features depend on the agent involved. Hyphae are usually present in tissue but may be difficult to demonstrate because of a tendency to form microcolonial granules.

Multiple species are involved

Trauma to bare feet injects the fungi

## ADDITIONAL READING

Kauffman CA. Sporotrichosis. *Clin Infect Dis* 1999;29:231–237. A review emphasizing clinical aspects, including excellent photographs.

Weitzman I, Summerbell RC. The dermatophytes. *Clin Microbiol Rev* 1995;8:240–259. This review includes genetics and therapy in addition to classic mycology and all the "tinea" clinical descriptions.

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CHAPTER 48

# *Candida, Aspergillus,* and Other Opportunistic Fungi

KENNETH J. RYAN

The fungi considered in this chapter are usually found as members of the normal flora or as saprophytes in the environment. With breakdown of host defenses they can produce disease ranging from superficial skin or mucous membrane infections to systemic involvement of multiple organs. The most common opportunistic infections are caused by the yeast *Candida albicans,* a normal inhabitant of the gastrointestinal and genital floras, and a mold, *Aspergillus,* commonly found in the environment. The diseases caused by *Candida, Aspergillus,* and other opportunistic fungi are summarized in Table 48–1.

## *CANDIDA*: GENERAL CHARACTERISTICS

*Candida* species grow as typical 4- to 6-$\mu$m, budding, round, or oval yeast cells (see Fig 45–1) under most conditions and at most temperatures. Under certain conditions, including those found in infection, they can form hyphae. Some species form chlamydoconidia in culture. *Candida* species identification is based on a combination of biochemical, enzymatic, and morphologic characteristics, such as carbohydrate assimilation; fermentation; and the ability to produce hyphae, germ tubes, and chlamydoconidia. Particular attention is given to the differentiation of *Candida albicans* from other species, because it is the most frequent cause of disease. It is also by far the best understood as to structure, metabolic activity, and pathogenesis.

Formation of hyphae and chlamydoconidia are distinguishing features

Carbohydrate assimilation and fermentation determine species

Most *Candida* species grow rapidly on Sabouraud's agar and on enriched bacteriologic media such as blood agar. Smooth, white, 2- to 4-mm colonies resembling those of staphylococci are produced on blood agar after overnight incubation. Aeration of cultures favors their isolation. The primary identification procedure involves presumptive differentiation of *C. albicans* from the more than 150 other *Candida* species with the germ tube test (see below). Germ tube–negative strains may be further identified biochemically or reported as "yeast not *C. albicans,*" depending on their apparent clinical significance.

Rapidly produce colonies resembling staphylococci

*C. albicans* produces germ tubes

TABLE 48–1

**Agents of Opportunistic Mycoses**

| Organism | Growth: Tissue | Growth: Culture at 25°C | Growth: Culture at 37°C | Source | Infection |
|---|---|---|---|---|---|
| *Candida* | Yeast (hyphae)[a] | Yeast (hyphae)[a] | Yeast | Endogenous | Skin, mucous membranes, urinary, disseminated |
| *Aspergillus* | Hyphae (septate) | Mold | Mold | Environment | Lung, disseminated |
| Zygomycetes[b] | Hyphae (nonseptate) | Mold | Mold | Environment | Rhinocerebral, lung, disseminated |

[a] Less common feature; pseudohyphae are produced as well.

[b] Such genera as *Absidia, Mucor,* and *Rhizopus*.

# *Candida albicans*

## MYCOLOGY

*C. albicans* grows in multiple morphologic forms, most often as a yeast with budding by formation of blastoconidia. *C. albicans* is also able to form hyphae triggered by changes in conditions such as temperature, pH, and available nutrients. When observed in their initial stages when still attached to the yeast cell, these hyphae look like sprouts and are called germ tubes (Fig 48–1A). Other elongated forms with restrictions at intervals are called **pseudohyphae** because they lack the parallel walls and septation of the true hyphae. There is evidence that these three forms have distinct stimuli and genetic regulation, making *C. albicans* a polymorphic fungus. Unless otherwise specified, the term **hyphae** is used here to encompass both the true and pseudohyphal forms. The hyphal form also develops characteristic terminal thick-walled **chlamydoconidia** under certain cultural conditions (Fig 48–1B).

Yeast, hyphae, and pseudohyphae are formed

Chlamydoconidia develop from hyphae in culture

The *C. albicans* cell wall is made up of a mixture of the polysaccharides mannan, glucan, and chitin alone or in complexes with protein. A fibrillar outer layer extending to the surface contains a number of distinct mannoproteins. The exact composition of the cell wall and surface components varies under different growth and morphologic conditions.

Cell wall includes surface mannoproteins

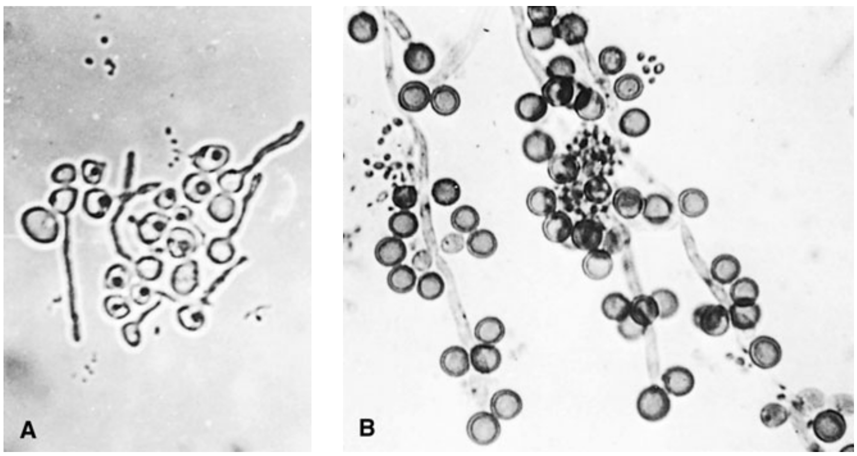


FIGURE 48–1
*Candida albicans*. **A.** When incubated at 37°C, *C. albicans* rapidly forms elongated hyphae called germ tubes. **B.** On specialized media, *C. albicans* forms thick-walled chlamydoconidia, which differentiate it from other *Candida* species. (*Reprinted with permission from Dr. E. S. Beneke and the Upjohn Company: Scope Publications, Human Mycoses.*)

# CANDIDIASIS

CLINICAL CAPSULE

Candidiasis occurs in localized and disseminated forms. Localized disease is seen as erythema and white plaques in moist skin folds (diaper rash) or on mucosal surfaces (oral thrush). It may also cause the itching and thick white discharge of vulvovaginitis. Deep tissue and disseminated disease are limited almost exclusively to the immunocompromised. Diffuse pneumonia and urinary tract involvement are especially common.

## EPIDEMIOLOGY

*C. albicans* is a common member of the oropharyngeal, gastrointestinal, and female genital flora. Infections are endogenous except in cases of direct mucosal contact with lesions in others (eg, through sexual intercourse). Although *C. albicans* is a common cause of nosocomial infections, the fungi are also derived more frequently from the patient's own flora than from cross-infection. Invasive procedures and indwelling devices may provide the portal of entry, and the number of available *Candida* may be enhanced by the used of antibacterial agents.

Infections are from endogenous flora

## PATHOGENESIS

Because *C. albicans* is regularly present on mucosal surfaces, disease implies a change in the organism, the host, or both. The change from the yeast to the hyphal form is strongly associated with enhanced pathogenic potential of *C. albicans*. In histologic preparations, hyphae are seen only when *Candida* starts to invade, either superficially or in deep tissues (see Fig 45–4). This switch can be controlled in vitro by the manipulation of environmental conditions, but it is not known what triggers the change in human disease. What is known is that the morphologic change is also associated with the appearance of a number factors associated with tissue adherence and digestion.

Shift from yeast to hyphae is associated with invasion

Switch is controlled by environmental conditions

*C. albicans* hyphae have the capacity to form strong attachments to human epithelial cells. A mediator of this binding may be a surface **hyphal wall protein** (Hwp1), which is found only on the surface of germ tubes and hyphae. This protein has amino acid sequences similar to those in the substrates of mammalian keratinocyte transaminases, which form cross-links between squamous epithelial specific proteins. This novel pathogenic strategy makes use of host enzymes to bind the pathogen to epithelial cells. Other mannoproteins that have similarities to vertebrate integrins may also mediate binding to components of the **extracellular matrix** (ECM), such as fibronectin, collagen, and laminin. Hyphae also secrete proteinases and phospholipases that are able to digest epithelial cells and probably facilitate invasion (Figs 48–2 and 48–3). There is also evidence that *C. albicans* may be able to induce its own phagocytosis by endothelial cells. Taken together, these factors represent a rich armamentarium of virulence factors all seemingly linked to the change from yeast to hyphal growth.

Surface mannoproteins bind to keratinocytes and ECM

Host enzymes link Hwp to tissue

Hyphae produce proteinases

*C. albicans* has protein surface receptors that bind the C3 component of complement in a manner similar to that of the receptors on neutrophils. C3 bound to the candidal surface by these receptors is thus oriented in a fashion that makes it unavailable for opsonization. Enhanced production of these receptors under various conditions, for example, elevated glucose concentration, is associated with resistance to phagocytosis by neutrophils.

Receptors bind C3 in an antiopsonic manner

Factors that allow *C. albicans* to increase its relative proportion of the flora (antibacterial therapy), that compromise the general immune capacity of the host (leukopenia or corticosteroid therapy), or that interfere with T-lymphocyte function (acquired immunodeficiency syndrome; AIDS) are often associated with local and invasive infection. The

Antimicrobics and immunosuppression increase risk

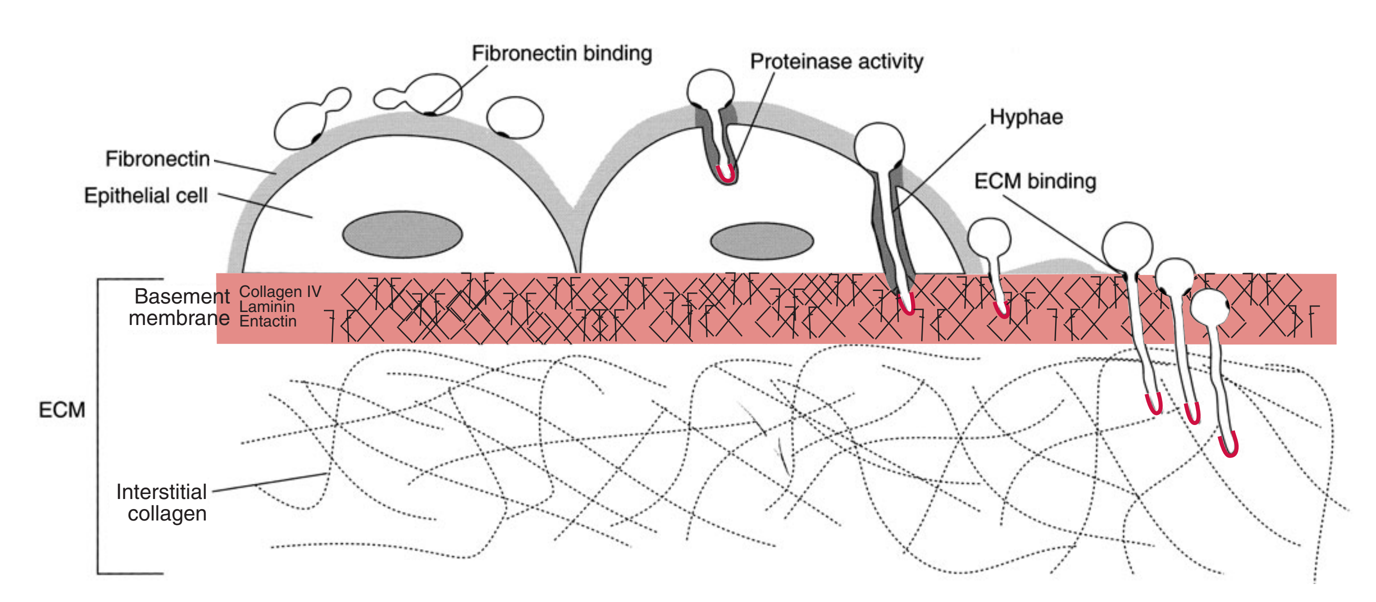


FIGURE 48–2
Pathogenesis of *Candida albicans* infections. Proposed mechanisms of *C. albicans* attachment and invasion are shown. Surface glucomannan receptor(s) on the yeast may bind to fibronectin covering the epithelial cell or to elements of the extracellular matrix (ECM) when the epithelial surface is lost or the *Candida* have invaded beyond it. Invasion is associated with formation of hyphae and production of proteinases, which may digest tissue elements.

Mechanical disruptions may provide access to ECM

disruptions of the mucosa associated with chronic disease and their treatments (indwelling devices, cancer chemotherapy) may enhance the invasion process by exposing *Candida* binding sites in the ECM. Diabetes mellitus also predisposes to *C. albicans* infection, possibly because of the known greater production of the surface mannoproteins in the presence of high glucose concentrations.

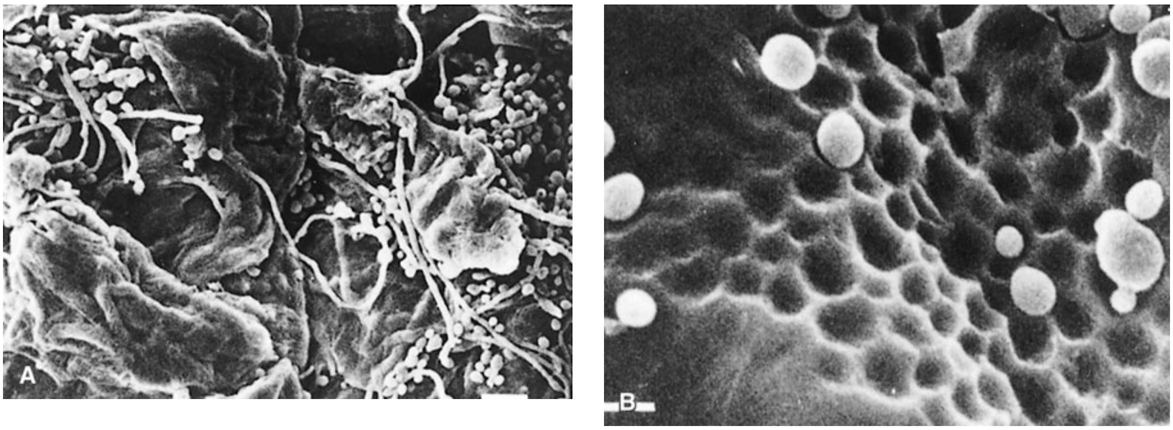


FIGURE 48–3
Invasiveness of *Candida albicans*. Two features of invasiveness are seen in these scanning electron micrographs taken from experiments with murine corneocytes. **A.** Both blastoconidia and mycelial elements are present. The mycelial elements spread over the surface and invade the cell cuticle. **B.** A *C. albicans* strain that produces a protease is seen producing cavity-like depressions in the cell surface. This action could play a role in invasion of the cell. (*Reprinted with permission of Thomas L. Ray and Candia D. Payne.* Infect Immun *1988;56:1945–1947, Figures 4,6B. Copyright American Society for Microbiology.*)

## IMMUNITY

Both humoral immunity and cell-mediated immunity are important in defense against *Candida* infections. Neutrophils are the primary first-line defense. Yeast forms of *C. albicans* are readily phagocytosed and killed when opsonized by antibody and complement. In the absence of specific antibody, the process is less efficient, but a naturally occurring antimannan IgG is able to activate the classical complement pathway and facilitate the alternate pathway. Hyphal forms may be too large to be ingested by polymorphonuclear neutrophils (PMNs), but they can still kill the fungi by attaching to the hyphae and discharging metabolites generated by the oxidative metabolic burst. A deficit in neutrophils or neutrophilic function is the most common correlate of serious *C. albicans* infection.

Opsonized yeast forms are killed by PMNs

Antimannan IgG activates complement

The association of chronic mucocutaneous candidiasis (see below) with a number of T-lymphocyte immunodeficiencies emphasizes the importance of this arm of the immune system in defense against *Candida* infections. The increased frequency of oral and vaginal candidiasis in AIDS patients suggests that even superficial infections involve T-lymphocyte–mediated immune responses (cell-mediated immunity [CMI]). In animal studies, *Candida* cell wall mannan has been shown to play an immunoregulatory function by downregulating cell-mediated immune responses. A possible explanation for the association between AIDS and *Candida* infection is the upregulation of CD4 receptors on monocytes by *Candida* products. As with other fungi, cytokine activation of macrophages enhances their ability to kill *C. albicans*. A favorable outcome appears to require the proper balance between $T_H1$- and $T_H2$-mediated cytokine responses. The cytokines associated with $T_H1$ (interleukin-2 [IL-2], IL-12, interferon-$\gamma$, tumor necrosis factor-$\alpha$) are correlated with enhanced resistance against infection where $T_H2$ responses (IL-4, IL-6, and IL-10) are associated with chronic disease.

Compromised CMI is associated with progressive infection

*Candida* mannan may downregulate CMI responses

Balance between $T_H1$ and $T_H2$ cytokines is necessary

# CANDIDIASIS: CLINICAL ASPECTS

## MANIFESTATIONS

Superficial invasion of the mucous membranes by *C. albicans* produces a white, cheesy plaque that is loosely adherent to the mucosal surface. The lesion is usually painless, unless the plaque is torn away and the raw, weeping, invaded surface is exposed. Oral lesions, called **thrush,** occur on the tongue, palate, and other mucosal surfaces as single or multiple, ragged white patches. A similar infection in the vagina, vaginal candidiasis, produces a thick, curd-like discharge and itching of the vulva. Although most women have at least one episode of **vaginal candidiasis** in a lifetime, a small proportion suffer chronic, recurrent infections. No general or specific immune defect has yet been linked to this syndrome.

White mucosal plaque is called thrush

Vaginitis may be recurrent

*C. albicans* skin infections occur in crural folds and other areas in which wet, macerated skin surfaces are opposed. For example, one type of diaper rash is caused by *C. albicans*. Other infections of the skin folds and appendages occur in association with recurrent immersion in water (eg, dishwashers). The initial lesions are erythematous papules or confluent areas associated with tenderness, erythema, and fissures of the skin. Infection usually remains confined to the chronically irritated area, but may spread beyond it, particularly in infants.

Macerated skin is a common site

In rare persons with specific defects in T cell–mediated immune defense against *Candida,* a chronic, relapsing form of candidiasis known as **chronic mucocutaneous candidiasis** develops. Infections of the skin, hair, and mucocutaneous junctions fail to resolve with adequate therapy and management. There is considerable disfigurement and discomfort, particularly when the disease is accompanied by a granulomatous inflammatory response. Although lesions may become extensive, they usually do not disseminate. To some degree this disease may represent a clinical example of immunologic tolerance. Cutaneous anergy to *C. albicans* antigens is commonly seen in these patients and is often reversed during antifungal chemotherapy, suggesting that it is due to chronic antigen excess.

Chronic mucocutaneous candidiasis is associated with specific T-cell defects

Esophagitis and intestinal candidiasis are similar to thrush

Inflammatory patches similar to those in thrush may develop in the esophagus with or without associated oral candidiasis. Painful swallowing and substernal chest pain are the most common symptoms. Extensive ulcerations, deformity, and occasionally perforation of the esophagus may ensue. In immunocompromised patients, similar lesions may also develop in the stomach, together with deep ulcerative lesions of the small and large intestine.

Urinary tract infections are ascending or hematogenous

Endophthalmitis appears as white cotton on retina

Infection of the urinary tract via the hematogenous or ascending routes may produce cystitis, pyelonephritis, abscesses, or expanding fungus ball lesions in the renal pelvis. The clinical findings in disseminated infections of the kidneys, brain, and heart are generally not sufficiently characteristic to suggest *C. albicans* over the bacterial pathogens, which more commonly produce infection of deep organs. *Candida* **endophthalmitis** has the characteristic funduscopic appearance of a white cotton ball expanding on the retina or floating free in the vitreous humor. Endophthalmitis and infections of other eye structures can lead to blindness.

## DIAGNOSIS

KOH and Gram smears of superficial lesions show yeast and hyphae

Lung involvement requires bronchoalveolar lavage

Superficial *C. albicans* infections provide ready access to diagnostic material. Exudate or epithelial scrapings examined by potassium hydroxide (KOH) preparations or Gram smear demonstrate abundant budding yeast cells; if associated hyphae are present, the infection is almost certainly caused by *C. albicans*. *C. albicans* is readily isolated from clinical specimens including blood if aerobic conditions are provided. Cultures from specimens such as sputum run the risk of contamination from the normal flora or a superficial mucous membrane lesion. A direct aspirate, biopsy, or bronchoalveolar lavage is often required to establish the diagnosis.

Endocarditis may require arterial cultures

Deep organ involvement is difficult to prove without a direct aspirate or biopsy. Even positive blood cultures must be interpreted with caution if they could represent colonization of intravenous catheters. *Candida* endocarditis represents a special diagnostic problem, because the yeasts seeding the blood from the valve may be filtered out in the capillary beds due to their large size. Arterial blood cultures may be required in this situation.

Immunodiagnostic procedures are not routine

Although many serologic tests have been developed for detection of *C. albicans* antibodies, none of the methods developed to date has the sensitivity or specificity needed for clinical diagnosis. Immunologic techniques for detection of circulating *Candida* cell components such as mannan show promise, but none are yet practical for clinical use.

## TREATMENT

Topical nystatin or azoles for superficial lesions

Amphotericin B, flucytosine, and azoles for invasive disease

*C. albicans* is usually susceptible to amphotericin B, nystatin, flucytosine, and the azoles. Superficial infections are generally treated with topical nystatin or azole preparations. Measures to decrease moisture and chronic trauma are important adjuncts in treating *Candida* skin infections. Deeper *C. albicans* infections may resolve spontaneously with elimination or control of predisposing conditions. Removal of an infected catheter, control of diabetes, or an increase in peripheral leukocyte counts is often associated with recovery without antifungal therapy. Persistent relapsing or disseminated candidiasis is treated with amphotericin B, flucytosine, fluconazole, or combinations of amphotericin B with other drugs. Fluconazole has been the most effective treatment for chronic mucocutaneous candidiasis.

# *Other Candida* Species

*C. tropicalis* is highly virulent

Species of *Candida* other than *C. albicans* produce infections in circumstances similar to those described previously, but do so less frequently. When contamination of an indwelling device is the portal of entry, the probability of infection by these other species increases. Little is known of the pathogenesis of these species with the exception of *Candida tropicalis*. Both experimental and clinical evidence indicate that *C. tropicalis* has virulence at least equal to that of *C. albicans*. *C. tropicalis* produces an extracellular proteinase similar to that of *C. albicans,* which may enhance its invasiveness.

*Candida glabrata* is another common species. This species is very small for a yeast (2- to 4-$\mu$m) and does not produce hyphae. It is a member of the normal gastrointestinal and genital flora. The most common infections are in the urinary tract, but deep tissue involvement and fungemia occur. The organisms are small enough to be confused with *Histoplasma capsulatum* in histologic preparations. Therapy is similar to that for *C. albicans* infections, although *C. glabrata* is more resistant to fluconazole.

*C. glabrata* is small for a yeast

Other species of *Candida,* which lack any distinguishing morphologic or clinical characteristics, may produce disease. Some of these fungi are inherently resistant to the antifungal azoles.

# *ASPERGILLUS*

## MYCOLOGY

Aspergillus species are rapidly growing molds with branching **septate hyphae** and characteristic arrangement of conidia on the conidiophore (Fig 48–4A). Fluffy colonies appear in 1 to 2 days and, by 5 days, may cover an entire plate with pigmented growth. Species are defined on the basis of differences in the structure of the **conidiophore** and the arrangement of the **conidia**. The most frequent in human infections are *Aspergillus fumigatus* and *Aspergillus flavus,* but others, such as *Aspergillus niger,* may be involved.

Species are based on arrangement of conidia on the conidiophore

## ASPERGILLOSIS

CLINICAL CAPSULE

Invasive aspergillosis is distinguished by its setting in immunocompromised individuals and its rapid progression to death. The typical patient is one with leukemia or under immunosuppression for a bone marrow transplant. The appearance of fever and a dry cough may be the only signs until pulmonary infiltrates are demonstrated radiologically. Until *Aspergillus* hyphae are demonstrated, almost any of the causes of pneumonia could be responsible.

### EPIDEMIOLOGY

*Aspergillus* species are widely distributed in nature and found throughout the world. They seem to adapt to a wide range of environmental conditions, and the heat-resistant conidia provide a good mechanism for dispersal. Like bacteria spores, the conida survive well in the environment and their inhalation is the mode of infection. Hospital air and air ducts have received attention as sources of nosocomial *Aspergillus* isolates. Occasionally, construction, remodeling, or other kinds of major environmental disruption have been associated with increased frequency of *Aspergillus* contamination, colonization, or infection.

Conidia may be spread by construction projects

### PATHOGENESIS

*Aspergillus* conidia are small enough to readily reach the alveoli when inhaled, but disease is rare in those without compromised defenses. Factors that aid the fungus in the initial stages are not known, but the ability of proteins on the surface of the conidia to bind fibrinogen and laminin probably contribute to adherence. Production of extracellular elastase, proteinases, and phospholipases has been associated with the more virulent species. The appearance of antibodies to these enzymes during and following invasive aspergillosis

Conidia bind to fibrinogen and laminin

Extracellular proteases may cause injury

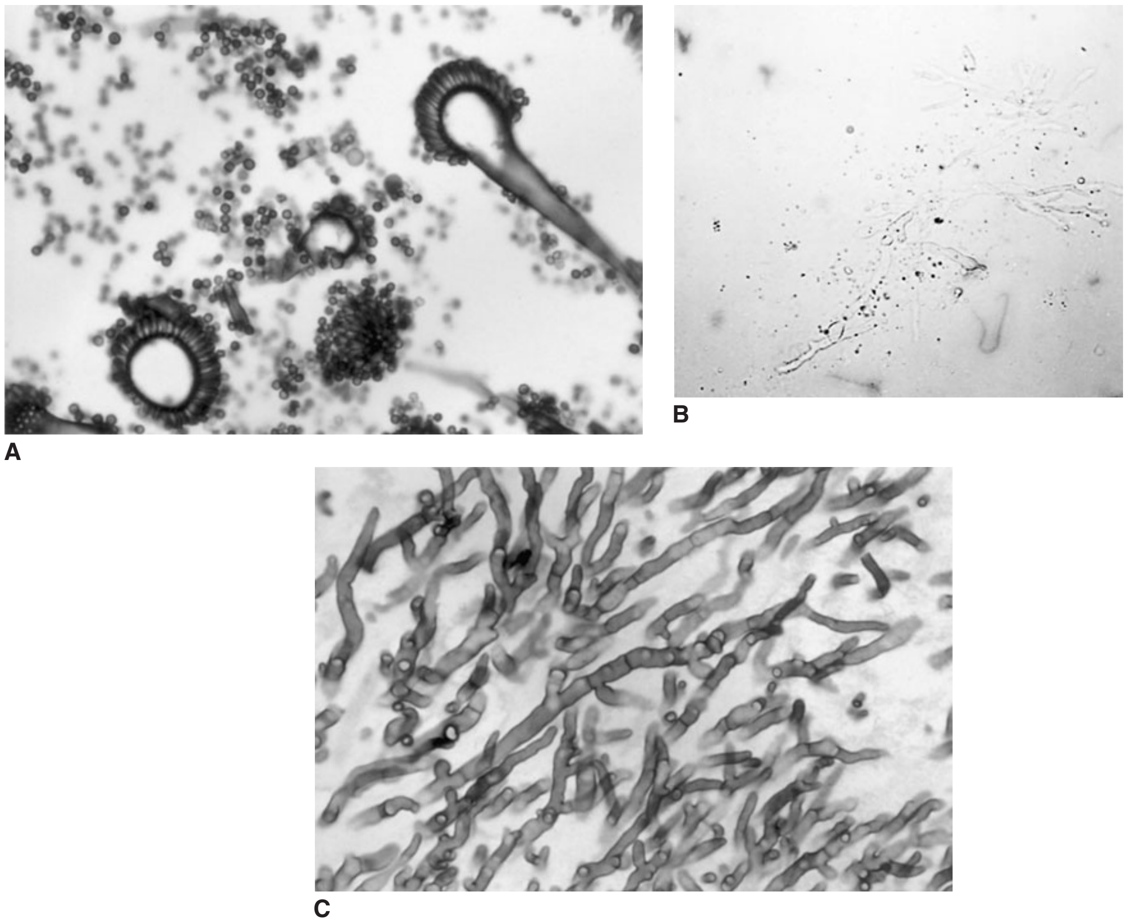


FIGURE 48–4

*Aspergillus*. **A.** This asexual conidium-forming structure is characteristic of *Aspergillus* species. The conidia are borne at the end of the finger-like extensions at the end of the conidiophore. These structures are rarely produced in vivo. **B.** This tissue aspirate mixed with KOH shows branching, septate hyphae. **C.** Histologic sections also show branching, septate hyphae, but because the conidia shown in **A** are not seen the findings are not diagnostic of *Aspergillus*. (*Reproduced with permission from Connor DH, Chandler FW, Schwartz DA, Manz HJ, Lack EE (eds).* Pathology of Infectious Disease, *Volume II. Stamford, CT: Appleton & Lange, 1997.*)

argues for their importance, but the pathogenic role of these enzymes remains to be demonstrated. Most species produce aflatoxins and other toxic secondary metabolites but their role in infection is also unknown.

## IMMUNITY

Alveolar macrophages kill conidia, and PMNs attack hyphae

Macrophages, particularly pulmonary macrophages, are the first line of defense against inhaled *Aspergillus* conidia phagocytosing and killing them by nonoxidative mechanisms. For the conidia that survive and germinate, PMNs become the primary defense. They are able to attach to the growing hyphae, generate an oxidative burst, and secrete reactive oxygen intermediates. Little is known of adaptive immunity in humans. Antibodies are formed but their protective value is unknown. Although AIDS patients do develop *Aspergillus* infections, the association with T-cell deficiencies is not strong enough to draw conclusions about their importance.

# ASPERGILLOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

*Aspergillus* can cause clinical allergies or occasional invasive infection. In both cases, the lung is the organ primarily involved. Allergic aspergillosis, which can be a mechanism of exacerbation in patients with asthma, is characterized by transient pulmonary infiltrates, eosinophilia, and a rise in *Aspergillus*-specific antibodies. These conditions follow direct inhalation of fungal elements or, more commonly, colonization of the respiratory tract. Areas of the bronchopulmonary tree with poor drainage because of underlying disease or anatomic abnormalities may serve as a site for growth of organisms and continuous seeding with antigen.

Allergic disease marked by eosinophilia and specific IgG

Invasive aspergillosis occurs in the settings of preexisting pulmonary disease (bronchiectasis, chronic bronchitis, asthma, tuberculosis) or immunosuppression. Colonization with *Aspergillus* can lead to invasion into the tissue by branching septate hyphae. In patients who already have a chronic pulmonary disease, mycelial masses can form a radiologically visible fungus ball (aspergilloma) within a preexisting cavity. Lung tissue invasion may penetrate blood vessels, causing hemoptysis or erosion into other structures with development of fistulas. Invasive disease outside the lung is rare unless patients are immunocompromised.

Highly invasive, including blood vessels

Fungus ball in cavities

An acute pneumonia may occur in severely immunocompromised patients, particularly those with phagocyte defects or depressed neutrophil counts due to immunosuppressive drugs. Multifocal pulmonary infiltrates expanding to consolidation are present with high fever. The prognosis is grave and dissemination to other organs common, which is not the case in immunocompetent hosts.

Pneumonia in immunocompromised host has grave prognosis

## DIAGNOSIS

*Aspergillus* is relatively easy to isolate and identify. Its rapidly spreading mold growth and all too frequent contamination of cultures cause it to be regarded by microbiologists as a kind of weed. The diagnostic problem is distinguishing contamination and colonization with *Aspergillus* from invasive disease. The diagnosis cannot be made for certain without the use of lung aspiration, biopsy, or bronchoalveolar lavage. With material directly from the lesion, the presence of large, branching, septate hyphae (Fig 48–4B and 48–4C) and a positive culture are diagnostic. Occasionally, the complete fruiting bodies are produced in vivo, creating a striking and diagnostic histologic picture (see Fig 48–4A). Serologic methods have been developed to demonstrate *Aspergillus* antibodies. Although these tests may be helpful in suggesting allergic aspergillosis, they have little value in invasive disease because anti-*Aspergillus* antibody is common in healthy persons.

Direct aspirate or biopsy is required to distinguish colonization from invasion

Serodiagnosis is useful only for allergic disease

## TREATMENT AND PREVENTION

Amphotericin B and itraconazole are the recommended antimicrobics for invasive aspergillosis. Neither can be considered particularly effective, because the mortality rate of invasive disease approaches 100%. In cases with pulmonary structural abnormalities and fungus balls, chemotherapy has little effect. Surgical removal of localized lesion is sometimes helpful, even in the brain. Construction of rooms with filtered air has been attempted to reduce exposure to environmental conidia.

Amphotericin B, itraconazole, and surgery are used for invasive disease

# ZYGOMYCETES AND ZYGOMYCOSIS

**Zygomycosis** (mucormycosis) is the term applied to infection with any of a group of zygomycetes, the most common of which are *Absidia, Rhizopus,* and *Mucor*. These fungi are ubiquitous saprophytes in soil and are commonly found on bread and many other

*Absidia, Rhizopus,* and *Mucor* are soil saprophytes

Immunocompromised hosts with diabetes are infected

foodstuffs. They occasionally cause disease in persons with diabetes mellitus and in immunosuppressed patients receiving corticosteroid therapy. Diabetic acidosis has a particularly strong association with zygomycosis.

Pulmonary disease is similar to other fungi

Sinus infections erode straight to the brain

Pulmonary or rhinocerebral disease is acquired by inhalation of conidia. The pulmonary form has clinical findings similar to those of other fungal pneumonias; the rhinocerebral form, however, produces a dramatic clinical syndrome in which agents of zygomycosis show striking invasive capacity. They penetrate the mucosa of the nose, paranasal sinuses, or palate, often resulting in ulcerative lesions. Once beyond the mucosa, they progress through tissue, nerves, blood vessels, fascial planes, and often the vital structures at the base of the brain. The clinical syndrome begins with headache and may progress through orbital cellulitis and hemorrhage to cranial nerve palsy, vascular thrombosis, coma, and death in less than 2 weeks.

Large ribbons of nonseptate hyphae are seen in tissues

The pathologic cerebral and pulmonary findings are distinctive: the zygomycetes involved all show ribbon-like **nonseptate hyphae** in tissue which are so large their branch points can be difficult to visualize. Conidia are not seen. As with *Aspergillus,* tissue biopsies are necessary to demonstrate the invasive hyphae, unless they can be seen on scrapings from palatal or nasal ulcers. For reasons that are obscure, cultures are sometimes negative, even those from tissue containing characteristic hyphae. Therapy involves control of underlying disease, amphotericin B, and occasionally surgery.

## ADDITIONAL READING

Denning DW. Invasive aspergillosis. *Clin Infect Dis* 1998;26:781–805. This review emphasizes the clinical aspects and illustrates them nicely.

Latge JP. *Aspergillus fumigatus* and aspergillosis. *Clin Microbiol Rev* 1999;12:310–350. A comprehensive review of mycologic and pathogenesis aspects, which also presents a nice view of diagnostic methods for the future.

Vazquez-Torrez A, Balish E. Macrophages in resistance to candidiasis. *Microbiol Mol Biol Rev* 1997;61:170–192. This review discusses all aspects of the immune response to *Candida* infection and their connection to the clinical forms of disease.

CHAPTER 49

# *Cryptococcus, Histoplasma, Coccidioides*, and Other Systemic Fungal Pathogens

KENNETH J. RYAN

The fungi discussed in this group cause a variety of infections, each ranging in severity from subclinical to progressive, debilitating disease. Most species are dimorphic, growing in the infectious mold form in the environment but switching to a yeast form in tissues to produce infection. They differ from the opportunistic fungi in their ability to cause disease in previously healthy persons, but the most serious disease still occurs in immunocompromised individuals. With the exception of *Cryptococcus neoformans,* each of these species is restricted to a geographic niche corresponding to the environmental habitat of the mold form of the species. None are transmitted from human to human. The major features of the systemic pathogens are summarized in Table 49–1.

## CRYPTOCOCCUS

### *Cryptococcus neoformans*

*Cryptococcus neoformans* (cryptococcus) is a yeast 4 to 6 $\mu$m in diameter that produces a characteristic **capsule** (Fig 49–1), extending the overall diameter to 25 $\mu$m or more. This capsule is unique among pathogenic fungi and is a complex polysaccharide polymer, the major component of which is **glucuronoxylomannan** (GXM). Capsule production varies by strain and with environmental conditions. It is repressed under environmental conditions and stimulated in the physiologic conditions found in tissues.

Yeasts produce large polysaccharide capsule in tissues

*C. neoformans* grows at 35 to 37°C on a variety of common media, including blood agar, chocolate agar, and Sabouraud's agar. Mucoid, bacteria-like colonies are produced

TABLE 49–1

**Features of Systemic Fungal Pathogens**

| Organism | Growth: Culture at 25°C | Growth: Culture at 37°C | Tissue | Source | Primary Disease | Disseminated Disease |
|---|---|---|---|---|---|---|
| *Cryptococcus neoformans* | Encapsulated yeast | Encapsulated yeast | Encapsulated yeast | Environment, worldwide | Pneumonia | Chronic meningitis |
| *Histoplasma capsulatum* | Mold, tuberculate macroconidia[a] | Small yeast | Small intracellular yeast[b] | Environment, US Midwest[c] | Pneumonia, hilar adenopathy | RES enlargement |
| *Blastomyces dermatitidis* | Mold[a] | Yeast | | Environment, US Midwest[c] | Pneumonia | Skin and bone lesions |
| *Coccidioides immitis* | Mold, arthroconidia | (Spherules)[d] | Spherules | Environment, Sonoran desert[c,e] | Valley fever | Pneumonia, meningitis, skin, bone |
| *Paracoccidioides brasiliensis* | Mold | Yeast, multiple blastoconidia | | Environment, Latin America | Pneumonia | Mucocutaneous, RES |

*Abbreviations:* RES, reticuloendothelial system (lymph nodes, liver, spleen, bone marrow).

[a] Micoconidia are formed but are not distinctive.

[b] Typically multiple yeast within macrophages.

[c] Ecologic "islands" are found throughout the Americas.

[d] It is difficult to grow the spherule phase in culture.

[e] In the United States includes parts of Arizona, California, Nevada, and western Texas.

Melanin and urease are produced in culture

in 2 to 3 days. In addition to the capsule, extracellular products include a urease enzyme and melanin pigment. The sexual state of *C. neoformans* places it in the Basidiomycetes, but this form has not been associated directly with disease.

## CRYPTOCOCCOSIS

CLINICAL CAPSULE

The primary disease caused by cryptococci is a chronic meningitis. The onset is slow, even insidious, with low-grade fever and headache progressing to altered mental state and seizures. In the cerebrospinal fluid (CSF) and in tissues, the inflammatory response is often remarkably muted. Most patients have some obvious form of immune compromise, although some show no demonstrable immune defect.

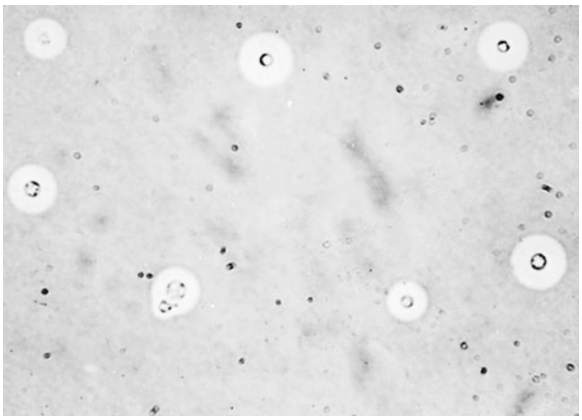

FIGURE 49–1
*Cryptococcus neoformans*. This India ink preparation was made by mixing cerebrospinal fluid containing cryptococci with India ink. The yeast cells can be seen within the clear space caused by the large polysaccharide capsule. (*Reprinted with permission from Dr. E. S. Beneke and the Upjohn Company: Scope Publications, Human Mycoses.*)

## EPIDEMIOLOGY

*C. neoformans* is found throughout the world, particularly in soil contaminated with pigeon or other bird droppings. The birds themselves are not ill. The cryptococci in the soil produce few or no capsules, which makes them more readily aerosolized. Inhalation of yeast cells stirred up from these sites is the presumed mode of transmission. Cases appear sporadically, with no particular occupational predisposition, including pigeon fanciers or in those who work with the organism in the laboratory. Cryptococcosis in immunocompromised patients occurs primarily in those with defects in T-lymphocyte function, particularly acquired immunodeficiency syndrome (AIDS), or in those treated with immunosuppressive agents (eg, steroids). Cryptococcal disease is the most common fungal infection seen in AIDS. Case-to-case transmission has not been documented.

Reservoir is soil contaminated with bird droppings

Inhalation of unencapsulated yeasts starts infection

## PATHOGENESIS

Following inhalation, the yeast begins to overproduce the polysaccharide capsule, which determines virulence. The capsule is antiphagocytic and has a number of other immunomodulating effects. The GXM is able to bind complement components while at the same time reducing the ability of polymorphonuclear neutrophils (PMNs) and macrophages to phagocytose and kill cryptococci. This may be due to the combination of the massive size of the capsule and the way in which it binds C3. There is also evidence that the capsule can interfere with antigen presentation and the development of T cell–mediated immune processes. This muting of the first lines of defense allows the organisms to multiply and eventually spread outside the lung. At this stage the organism has a strong affinity for the central nervous system (CNS), possibly due to its C3 binding and the relatively low levels of complement found there.

Antiphagocytic capsule is produced after inhalation

GXM binds C3 and interferes with antigen presentation

Cryptococci produce enough capsule that the GXM can be readily detected in the blood and other body fluids. This circulating polysaccharide is able to downregulate immune responses, particularly the development of protective $T_H1$-type mediators and suppression of the specific antibody response. These modulations may be either antigen specific or cause a general suppression of key immune functions, such as leukocyte migration. Cryptococci are also able to oxidize exogenous catecholamines to produce melanin, a process that may protect them from the oxidative injury of phagocytes.

Circulating antigen depresses humoral and cell-mediated immunity

Melanin production provides oxidative protection

Tissue reaction to *C. neoformans* varies from little or none to purulent or granulomatous. Many cases of pulmonary, cutaneous, and even meningeal cryptococcal infection show a remarkable paucity of inflammatory cells. This certainly fits for a fungus that not only blocks its own phagocytosis but is able to downregulate multiple aspects of the immune response.

Tissue reaction is often minimal

## IMMUNITY

In immunocompetent persons, alternate pathway binding of complement by the capsule is probably sufficient for opsonophagocytosis. The capsule is not particularly antigenic, and anticryptococcal antibodies are not usually detected in the course of infection. When formed, the classical pathway can play a role in opsonization, but this mechanism is not believed to play a strong role in immunity. Anticryptococcal antibody and complement do not directly damage the organism but may be a key component in the development of cellular host defense mechanisms and the clearance of circulating antigen.

Alternate pathway is more important than classical

Animal studies and the strong clinical association of cryptococcosis with T-cell defects indicate that T lymphocyte–mediated immune responses are crucial to the outcome of infection. Cryptococci phagocytosed by macrophages may not be killed, and cytokine activation is needed to complete the clearing of the organisms. Patients with cryptococcosis who have no known immune defects often have subnormal cellular immune functions as measured by their lymphocyte-mediated responses to cryptococcal and other antigens. Clinical recovery in such cases is associated with return of cellular immune functions.

T-cell responses are crucial to outcome

Cell-mediated immunity may return with recovery

## CRYPTOCOCCOSIS: CLINICAL ASPECTS

### MANIFESTATIONS

Meningitis is insidious and chronic

Course is more rapid with AIDS

Meningitis is the most commonly recognized form of cryptococcal disease; it usually has a slow, insidious onset with relatively nonspecific findings until late in its course. Intermittent headache, irritability, dizziness, and difficulty with complex cerebral functions appear over weeks or months with no consistent pattern. Behavioral changes have been mistaken for psychoses. Fever is usually, but not invariably, present. Seizures, cranial nerve signs, and papilledema may appear later in the clinical course, as may dementia and decreased levels of consciousness. A more rapid course may be seen in AIDS patients, 5 to 15% of who become infected with *C. neoformans*.

Cryptococcal pneumonia is usually asymptomatic

Cryptococcal pneumonia is often asymptomatic or mild. Sputum production is minimal, and no findings are sufficiently specific to suggest the etiology. Skin and bone are the sites most frequently involved in disseminated disease; skin lesions are sometimes the presenting sign and are often remarkable for their lack of inflammation. The diagnosis is sometimes made when lesions are biopsied as suspected neoplasms.

### DIAGNOSIS

Cells and glucose depression in CSF may be minimal

India ink prep is positive in 50% of cases

Typical cerebrospinal fluid (CSF) findings in cryptococcal meningitis are increased pressure, pleocytosis (usually $\geq$100 cells) with predominance of lymphocytes, and depression of glucose levels. In some cases, one or all of these findings may be absent, yet cryptococci are isolated on culture. Cryptococcal capsules are demonstrable in CSF in roughly 50% of cases by mixing centrifuged sediment with **India ink** and examining the mixture under the microscope (see Fig 49–1). Some experience is necessary to avoid confusion of lymphocytes with cryptococci. *C. neoformans* stains poorly or not at all with routine histologic stains; thus, it is easily missed unless special fungal stains are used.

Few cryptococci may be present in CSF

GXM is detectable in CSF and serum

In the isolation of *C. neoformans,* the volume of CSF sampled is important. The number of organisms present may be small enough to require a substantial volume of fluid (>30 mL) to yield a positive culture. If cryptococcosis is suspected and cultures are negative, detection of the GXM polysaccharide antigen in the CSF or serum by latex agglutination or enzyme immunoassay methods is recommended. These tests are very sensitive and specific, and their quantitation has prognostic significance. A rising antigen level indicates progression and a declining titer is a favorable sign.

### TREATMENT

Amphotericin, fluconazole, and flucytosine used in combination

Amphotericin B (with or without flucytosine) or fluconazole is the usual treatment for systemic cryptococcal disease. Flucytosine use alone is limited by development of resistance during therapy. Although three fourths of persons with meningitis respond to treatment, a significant portion suffer relapses after antifungal therapy is stopped; many become chronic and require repeated courses of therapy. One half of those cured have some kind of residual neurologic damage.

# *HISTOPLASMA*

## *Histoplasma capsulatum*

*Histoplasma capsulatum* is a dimorphic fungus that grows in the yeast phase in tissue (Fig 49–2A) and in cultures incubated at 37°C. The mold phase grows in cultures incubated at 22 to 25°C and as a saprophyte in soil. The yeast forms are small for fungi (2 to

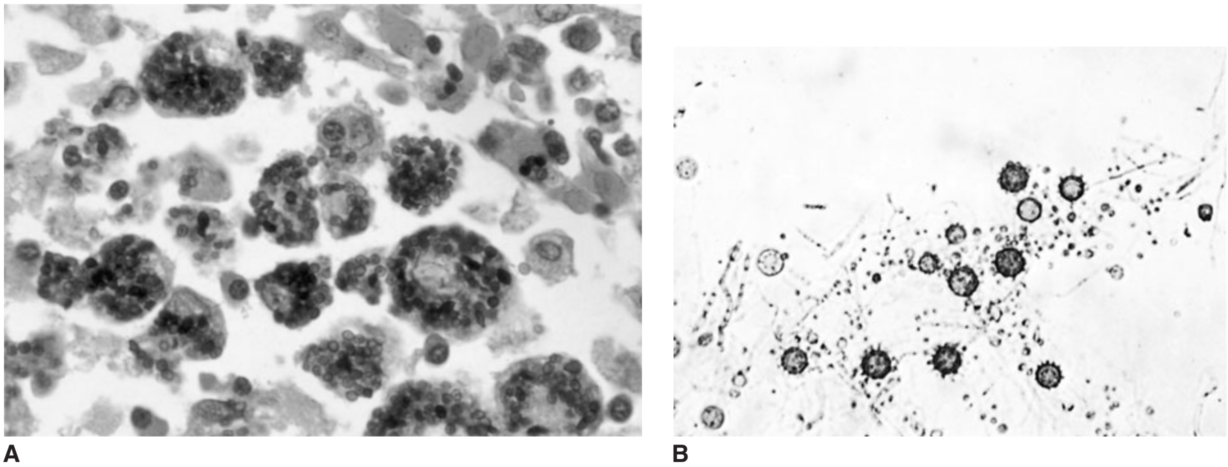


FIGURE 49–2
*Histoplasma capsulatum*. **A.** Multiple organisms are stuffed within the cytoplasm of alveolar macrophages in the lung. (*Reproduced with permission from Connor DH, Chandler FW, Schwartz DA, Manz HJ, Lack EE (eds).* Pathology of Infectious Disease, *Volume II. Stamford, CT: Appleton & Lange, 1997.*) **B.** The mold is shown with characteristic tuberculate macroconidia. (*Reprinted with permission from Dr. E. S. Beneke and the Upjohn Company: Scope Publications, Human Mycoses.*)

4 $\mu$m) and reproduce by budding (blastoconidia). The mycelia are septate and produce **microconidia** and macroconidia. The diagnostic structure is termed the **tuberculate macroconidium** because of its thick wall and radial, finger-like projections (see Fig 49–2B). Growth is obtained on blood agar, chocolate agar, and Sabouraud's agar, but may take many weeks. A sexual stage has now been discovered (*Ajellomyces capsulatum*), but the asexual name continues to be used in the medical literature. The designation *H. capsulatum* is actually a misnomer, because no capsules are formed. It comes from the halos seen around the yeasts in tissue sections, which are caused by a shrinkage artifact of routine histologic methods.

Small dimorphic fungus producing tuberculate macroconidia

Growth may take weeks

## DIMORPHISM

The morphologic and physiologic events associated with conversion from the mold to the yeast phase of *H. capsulatum* have been extensively studied. They are understandably complex given the dramatic change of milieu encountered by the fungus when its mold conidia float from their soil habitat to the pulmonary alveoli. Conversion to the yeast phase is then triggered by the host temperature (37°C) and possibly by other aspects of the new environment. In vitro studies show that the earliest events in this shift from the mold to yeast form involve induction of the **heat shock response** and uncoupling of oxidative phosphorylation. These are followed by a shutdown of RNA synthesis, protein synthesis, and respiratory metabolism. The cells then pass through a metabolically inactive state, emerging with enhanced enzymatic capacities involving sulfhydryl compounds (eg, cysteine, cystine) that are exclusive to the yeast stage. In the yeast stage, there is recovery of mitochondrial activity and synthetic capacity, but a new constellation of oxidases, polymerases, proteins, cell wall glucans, and other compounds are present.

Shift from mold to yeast begins with heat shock response

Metabolic shift is toward sulfhydryl compounds in yeast form

Dimorphism in fungi is reversible, a feature that distinguishes it from developmental processes such as embryogenesis seen in higher eukaryotes. The importance of the conversion to virulence of *Histoplasma* is shown by animal studies using strains biochemically blocked from converting to the yeast phase. They neither produce disease nor persist in the host. To the extent known, these features are similar in the other dimorphic fungi.

Dimorphism is reversible and linked to virulence

# HISTOPLASMOSIS

CLINICAL CAPSULE

Histoplasmosis is limited to the endemic area, where the vast majority of cases are asymptomatic or show only a fever and cough. If affected individuals are seen by a physician, a pulmonary infiltrate and hilar adenopathy may or may not be evident on a radiograph. Progressive cases show extension in the lung or enlargement of lymph nodes, liver, and spleen.

## EPIDEMIOLOGY

Microconidia are infectious

Mold grows in humid soil and bird droppings

Central US states have high prevalence

*H. capsulatum* grows in soil under humid climatic conditions, particularly soil containing bird or bat droppings. Inhalation of the mold microconidia, which are small enough (2 to 5 $\mu$m) to reach the terminal bronchioles and alveoli, is believed to be the mode of infection. The organism has a worldwide distribution but is particularly prevalent in certain temperate, subtropical, and tropical zones. In the United States, the greatest concentration by far is in the areas drained by the Ohio and Mississippi Rivers (see Fig 49–7). Over 50% of the residents of states in this area show evidence of previous infection, and in some locales, up to 90% of those have positive skin tests. Disturbances of bird roosts, bat caves, and soil have been associated with point source outbreaks. Persons in endemic areas whose employment (agriculture, construction) or avocation (spelunkers) brings them in contact with these sites are at increased risk. The infection is not transmitted from person to person. Disease is more common in men but there are no racial or ethnic differences in susceptibility.

## PATHOGENESIS

Reticuloendothelial system is focus of infection

Grows in macrophages by controlling lysosomal pH

The hallmark of histoplasmosis is infection of the lymph nodes, spleen, bone marrow, and other elements of the reticuloendothelial system with intracellular growth in phagocytic macrophages. The initial infection is pulmonary, through inhalation of infectious conidia, which convert to the yeast form in the host. They attach to CD18 integrin receptors and are readily phagocytosed by macrophages and PMNs. Inside phagocytes, they continue to multiply in the cytoplasm, surviving the combined effects of the oxidative burst and phagolysosomal fusion. Key features in this survival are the ability of *H. capsulatum* to capture iron and calcium from the macrophage and to modulate phagolysosomal pH. The acidic pH required for optimal killing effect in the lysosome is elevated by *H. capsulatum* toward the neutral range (pH 6.0 to 6.5).

Lymphatic spread and reactivation are similar to tuberculosis

With continued growth, there is lymphatic spread and development of a primary lesion similar to that seen in tuberculosis (see Chapter 28). The extent of spread to the reticuloendothelial system within macrophages during primary infection is unknown, but such spread is presumed to occur. The vast majority of cases never advance beyond the primary stage, leaving only a calcified node as evidence of infection. Old lesions may reactivate in a small proportion of cases.

Granulomatous response seen in liver, spleen, and bone marrow

Pathologically, granulomatous inflammation with necrosis is prominent in pulmonary lesions, but *H. capsulatum* may be difficult to detect, even with special fungal stains. Extrapulmonary spread involves the reticuloendothelial system, with enlargement of the liver and spleen. Numerous organisms within macrophages may be found in these organs, in lymph nodes, or in bone marrow (see Fig 49–2A).

## IMMUNITY

Histoplasmin skin test demonstrates delayed hypersensitivity

Infection with *H. capsulatum* is associated with the development of cell-mediated immunity, as demonstrated by a positive delayed hypersensitivity skin test to a mycelial antigen called **histoplasmin.** Infection is believed to confer long-lasting immunity, the most important component of which is CD4+ T lymphocyte mediated. In experimental infections, macrophages activated by T lymphocyte–derived cytokines are able to inhibit intracellular growth of *H. capsulatum* and thus control the disease. Neither B cells nor antibody have a significant influence on resistance to reinfection. Immunocompromised persons, particularly those with T lymphocyte–related defects, are unable to stop growth of the organism and tend to develop progressive, disseminated disease.

Immunity is derived from T-cell activation of macrophages

# HISTOPLASMOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

Most cases of *H. capsulatum* infection are asymptomatic or show only fever and cough for a few days or weeks. Mediastinal lymphadenopathy and slight pulmonary infiltrates may be seen on x-rays. The histoplasmin skin test becomes positive after about 3 weeks. More severe cases may have chills, malaise, chest pain, and more extensive infiltrates, which usually resolve nonetheless. A residual nodule may continue to enlarge over a period of years, causing a differential diagnostic problem with pulmonary neoplasms. Progressive pulmonary disease occurs in a form similar to that of pulmonary tuberculosis, including the development of cavities, with sputum production, night sweats, and weight loss. The course is chronic and relapsing, lasting many months to years.

Most cases are asymptomatic or with fever and cough

Progressive pulmonary disease shows cavities and weight loss

Disseminated histoplasmosis generally appears as a febrile illness with enlargement of reticuloendothelial organs. The CNS, skin, gastrointestinal tract, and adrenal glands may also be involved. Painless ulcers on mucous membranes are a common finding. The course is typically chronic, with manifestations that depend on the organs involved. For example, chronic bilateral adrenal failure (Addison's disease) may develop when the adrenal glands are involved.

Dissemination involves reticuloendothelial organs, mucous membranes, and adrenal glands

## DIAGNOSIS

In most forms of pulmonary histoplasmosis, the diagnostic yield of direct examinations or culture of sputum is low. In disseminated disease, blood culture or biopsy samples of a reticuloendothelial organ are the most likely to contain *Histoplasma*. Bone marrow culture has the highest yield. Because of their small size, the yeast cells are difficult to see in potassium hydroxide (KOH) preparations, and their morphology is not sufficiently distinctive to be diagnostic. Selective fungal stains such as methenamine silver demonstrate the organism but may not differentiate it from other yeasts. Hematoxylin and eosin (H&E)–stained tissue or Wright-stained bone marrow often demonstrates the organisms in their intracellular location in macrophages (see Fig 49–2). Specimens must be examined carefully under high magnification. Identification of culture isolates requires demonstration of the typical conidia and dimorphism. Demonstration of specific mycelial antigens by immunodiffusion (exoantigen test) may be used in place of dimorphism demonstration. Nucleic acid probes have been developed for culture identification.

Blood and bone marrow examination require special stains

Immunodiffusion and probes used with cultures

Antibodies can be detected during and following infection, but their usefulness in the endemic area is limited by false-negative results and cross-reactions in patients with blastomycosis. Rising antibody titers are suggestive of dissemination or relapse. The histoplasmin skin test is useful for epidemiologic studies but is not used for diagnosis or management of individual cases. Cultural isolation or clear histologic demonstration is necessary for a firm diagnosis. A circulating polysaccharide antigen has been demonstrated in serum and urine by enzyme immunoassay (EIA) in more than 90% of patients with disseminated disease.

Culture is required for firm diagnosis

EIA detects circulating antigen

## TREATMENT

Primary infections and localized lung lesions usually resolve without treatment. Amphotericin B remains the treatment of choice, but its toxicity limits its use to cases of extensive disease such as progressive pulmonary and disseminated histoplasmosis. Itraconazole and ketoconazole have been effective for treatment and for suppression in AIDS patients with

Amphotericin B and itraconazole

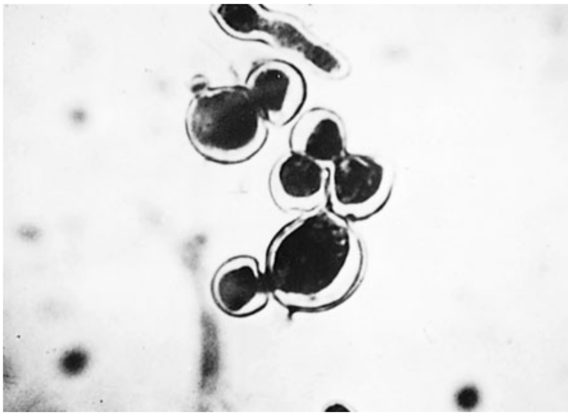

FIGURE 49–3
*Blastomyces dermatitidis.* Large thick-walled yeast cells are shown. Blastoconidia retain a broad attachment to the mother cell before separating. (*Reprinted with permission from Dr. E. S. Beneke and the Upjohn Company: Scope Publications, Human Mycoses.*)

histoplasmosis. In some cases amphotericin B treatment may be followed with a course of itraconazole.

# BLASTOMYCES

## Blastomyces dermatitidis

Large yeast cells have broad-based buds

Mold has small oval conidia-like *Histoplasma*

*Blastomyces dermatitidis* is a dimorphic fungus with some characteristics similar to those of *Histoplasma*. Growth develops in the yeast phase in tissues and in cultures incubated at 37°C. The yeast cells are typically larger (8–15 mm) than those of *H. capsulatum,* with broad-based buds and a thick wall (Fig 49–3). The mold phase appears in culture at 25°C. Hyphae are septate and produce round to oval conidia sufficiently similar to the microconidia produced by *H. capsulatum* to cause confusion between the two in young cultures. Although older cultures may produce chlamydoconidia, *B. dermatitidis* produces no structure as distinctive as the tuberculate macroconidium of *Histoplasma*.

## BLASTOMYCOSIS

CLINICAL CAPSULE

Most clinical features of blastomycosis are similar to histoplasmosis. Patients are asymptomatic or have only mild fever and cough unless the disease progresses outside the lung. Skin lesions are the most common manifestation of disseminated disease, not reticuloendothelial organ involvement.

### EPIDEMIOLOGY

Geographic distribution is similar to *Histoplasma*

Cases of blastomycosis follow a geographic distribution and conditions for maturation of conidia in the soil, which are similar to that of histoplasmosis (see Fig 49–7). Most infections occur in the middle and eastern portions of North America, but cases have been reported worldwide. The lack of a specific skin test limits study of the endemic area.

### PATHOGENESIS

Much less is known about blastomycosis than the more common systemic mycoses, such as histoplasmosis and coccidioidomycosis. The lower frequency of disseminated infections and the nonspecificity of skin and serologic tests are partly responsible for this lack of information. Much of what is believed to be true of blastomycosis is based on analogy with histoplasmosis.

The primary infection is pulmonary after inhalation of conidia, which develop in soil. Surface glucans and a glycoprotein adhesin (BAD1) have been identified, which bind the fungi to receptors on host cells, macrophages (CR3 and CD14), and the extracellular matrix. A mixed inflammatory response results, which ranges from neutrophil infiltration to well-organized granulomas with giant cells. The organisms grow in tissue as large yeasts with thick double walls with blastospores attached. A significant difference from *Histoplasma* is that the yeast cells are primarily extracellular rather than within macrophages. This may be due to their relatively large size, but there is little to suggest that *B. dermatitidis* shares the propensity for intracellular parasitism that is characteristic of *H. capsulatum*.

Surface adhesin binds to host cells

Large yeast are primarily outside cells

## IMMUNITY

The principal host defense mechanisms against *B. dermatitidis* have not been clearly defined. The fungal cells activate the complement system by both the classical and alternate pathways, and antibodies directed against a glucan component of the cell wall have been identified. These antibodies decline as the infection resolves. As with other fungi, T lymphocyte–mediated responses appear to be the most important determinants of immunity. Macrophages activated with cytokines have enhanced capacity to kill *B. dermatitidis*.

Complement, antibody, and cell-mediated immunity are involved

# BLASTOMYCOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

Because mild cases are difficult to diagnose, most infections are recognized at advanced or disseminated stages of the disease. This problem was also posed by the other systemic mycoses before the development of sensitive and specific diagnostic procedures. Pulmonary infection is evidenced by cough, sputum production, chest pain, and fever. Hilar lymphadenopathy may be present, as may nodular pulmonary infiltrates with alveolar consolidation. The total picture may mimic a pulmonary tumor, tuberculosis, or some other mycosis. Skin lesions are common and were once considered a primary form of the disease. In contrast to histoplasmosis, lesions develop on exposed skin; mucous membrane infection is uncommon. Extensive necrosis and fibrosis may produce considerable disfigurement. Bone infection has features similar to those of other causes of chronic osteomyelitis. The urinary and genital tracts are the most commonly affected visceral sites; the prostate is especially prone to infection.

Pulmonary blastomycosis is similar to other mycoses

Skin lesions are on exposed surfaces

## DIAGNOSIS

Direct demonstration of typical large yeasts with broad-based buds (blastoconidia) in KOH preparations is the most rapid means of diagnosis. Biopsy specimens also have a high yield, and the organisms are visible with either H&E or special fungal stains. *B. dermatitidis* grows on routine mycologic media, but culture may take as long as 4 weeks. Conidia are not particularly distinctive, and demonstration of dimorphism and typical yeast morphology is essential to avoid confusion with other fungi. The immunodiffusion test is particularly useful in differentiating cultures from *Histoplasma*. Serologic tests are available but may be negative in up to 50% of cases. Skin tests are no longer available.

KOH and biopsy show budding yeast

Culture takes weeks and conidia not distinctive

## TREATMENT

Although amphotericin B is the preferred therapy, it is used only for progressive or disseminated disease. As with other systemic mycoses, response to treatment is slow, and relapse is common. Itraconazole, ketoconazole, and fluconazole have been effective in nonmeningeal

Amphotericin B and azoles are effective

cases and for suppression in AIDS. These azoles are considered alternatives to amphotericin in immunocompetent patients if the disease is not severe.

# COCCIDIOIDES

## Coccidioides immitis

Dimorphism involves unique spherule

Spherules differentiate to form and release endospores

*Coccidioides immitis* is also a dimorphic fungus, but instead of a yeast phase, a large (12- to 100-$\mu$m), distinctive, round-walled **spherule** (Fig 49–4A and C) is produced in the invasive tissue form. This structure is unique among the pathogenic fungi. Its formation takes place in a process illustrated in Figure 49–5. Spherule development requires simultaneous invagination of the fungal membrane (plasmalemma) and production of new cell wall to form the large multicompartmental structure. The compartments differentiate into uninucleate structures called **endospores,** each with a thin wall layer. Multiple endospores develop within each spherule and the entire structure is surrounded by an extracellular matrix. The spherule eventually ruptures, releasing 200 to 300 endospores (Fig 49–6) each of which can differentiate into another spherule.

In alkaline soils and in culture, *C. immitis* grows only as a mold regardless of temperature. Growth becomes visible in 2 to 5 days. The hyphae are septate and produce

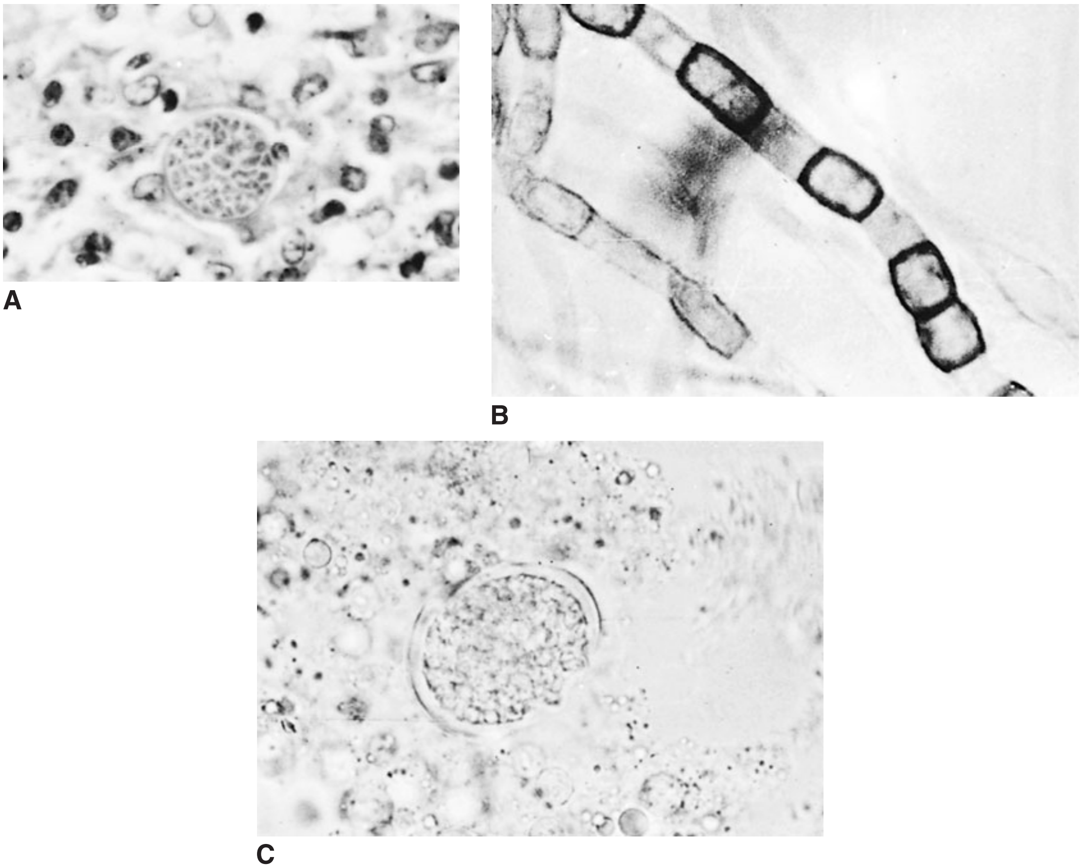


FIGURE 49–4

*Coccidioides immitis*. **A.** Tissue with thick-walled spherule containing multiple endospores. **B.** Mold phase with septate hyphae and arthroconidia. **C.** KOH preparation of sputum showing thick-walled spherule, which has just burst. (*Reprinted with permission from Dr. E. S. Beneke and the Upjohn Company: Scope Publications, Human Mycoses.*)

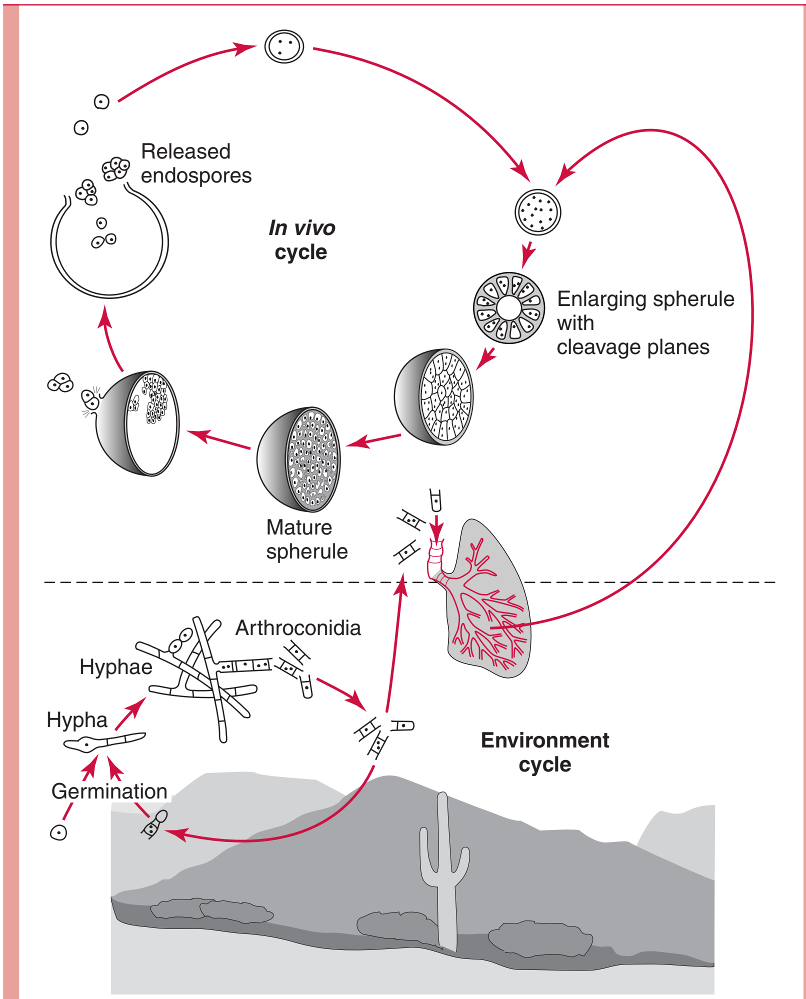


FIGURE 49–5
Life cycle of *Coccidioides immitis.* The nature cycle takes place in desert climates with modest rainfall. Hyphae differentiate into arthroconidia which break loose and may be suspended in the air. Soil disruptions and wind facilitate spread and the probability of inhalation into human lungs. In the human host environment in vivo differentiation produces cleavage planes and eventually huge spherules. The spherules rupture releasing endospores which can then repeat the in vivo cycle.

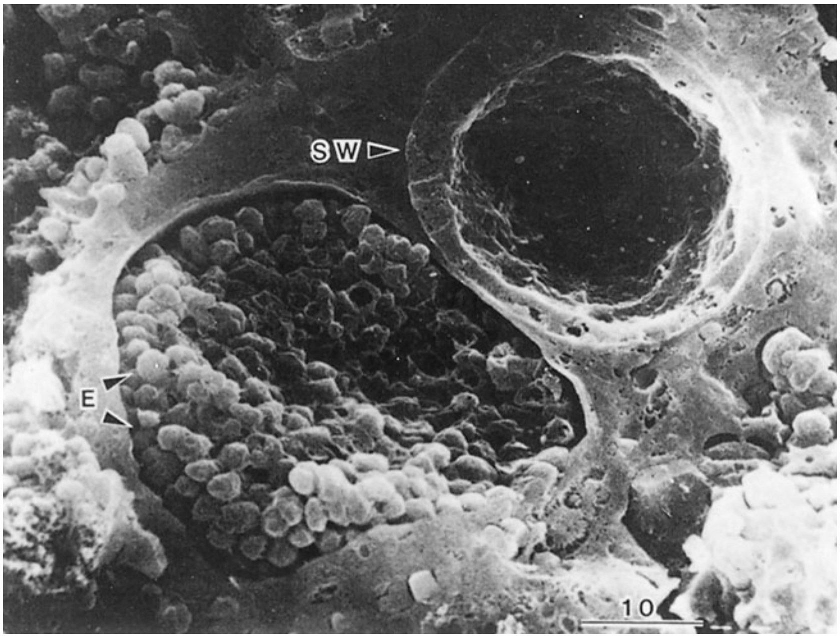


FIGURE 49–6
*Coccidioides immitis*. This electron micrograph of infected mouse lung shows a spherule filled with endospores (E) and one that has discharged its endospores into the surrounding tissue. Note the thickness of the spherule wall (SW). (*Reprinted with permission from Drutz DJ, Huppert M.* J Infect Dis *1983;147:379, Figure 7. Copyright University of Chicago Publisher.*)

Barrel-shaped arthroconidia are highly infectious

thick-walled, barrel-shaped **arthroconidia** (see Fig 49–4B), which are the infectious unit in nature and highly infectious when they develop in the laboratory. Spherules have been produced from arthroconidia in vitro under specialized conditions.

# COCCIDIOIDOMYCOSIS

CLINICAL CAPSULE

Acute primary infection with *C. immitis* is either asymptomatic or presents as a complex called **valley fever** by residents of the endemic areas. Valley fever includes fever, malaise, dry cough, joint pains, and sometimes a rash. There are few physical or radiologic findings, but the illness persists for weeks. Disseminated disease involves lesions in the bones, joints, skin, and a progressive chronic meningitis.

## EPIDEMIOLOGY

Geographically restricted to Sonoran desert

High proportion of locals have been infected

Coccidioidomycosis is the most geographically restricted of the systemic mycoses, because *C. immitis* grows only in the alkaline soil of semiarid climates known as the Lower Sonoran life zone (see Fig 49–7). These areas are characterized by hot, dry summers, mild winters with few freezes, and annual rainfall of about 10 inches during brief rainy seasons. Areas with these conditions are found scattered throughout the Americas, some as ecologic "islands." The primary endemic zones in the United States are in Arizona, Nevada, New Mexico, western Texas, and the arid parts of central and southern California. Persons living in the endemic areas are at high risk of infection, although disease is much less common. Positive skin test rates of 50 to 90% occur in longtime residents of highly endemic areas. Coccidioidomycosis is not transmissible from person to person.

Arthroconidia can be spread by dust storms

Rainfall pattern influences attack rate

Infection cannot be acquired without at least visiting an endemic area, although some interesting examples of the endemic zone itself paying a visit have been recorded. One such anecdote involves a gas station attendant with coccidioidomycosis whose only contact with an endemic area was changing a flat tire on a truck from California. In 1978, a storm originating in Bakersfield, California (endemic zone) carried a thick coat of dust all the way to San Francisco. This was followed by cases of coccidioidomycosis in persons who had never left the Bay Area. In 1992, a tenfold increase in disease in California followed an unusually wet winter in which the storms created a drought–rain–drought pattern just the right for growth of the mold (and wildflowers). When the Sonoran desert blooms, an arthroconidium "crop" is not far behind.

## PATHOGENESIS

Arthroconidial wall resists phagocytosis

Spherules produce endospores with extracellular matrix

Inhaled arthroconidia are small enough (2 to 6 $\mu$m) to bypass the defenses of the upper tracheobronchial tree and lodge in the terminal bronchioles. Human monocytes can ingest and kill some arthroconidia on initial exposure, although the outer portion of the wall of the arthroconidium has antiphagocytic properties, which persist in the early stages of spherule development. Surviving arthroconidia convert to the spherule stage, which begins its slow growth to a size that makes effective phagocytosis difficult. Although PMNs are able to digest the spherule wall, their access appears to be restricted by the extracellular matrix surrounding it. The young endospores are released in packets that include the extracellular matrix derived from the parent spherule, which may protect them until they develop into new spherules.

Proteases and SOW may be linked to virulence

A number of proteases found in the conidial cell wall or in spherules have been proposed as *C. immitis* virulence factors. In addition to their role in the fungal life cycle, some of these enzymes attack host substrates such as collagen, elastin, and immunoglobulins, but no direct specific contribution to disease has been defined. Components of the spherule outer wall (SOW) have been linked to virulence in animals and to strong humoral and cellular immune responses in humans.

## IMMUNITY

Lifelong immunity to coccidioidomycosis clearly develops in the vast majority of those who become infected. This immunity is associated with strong polymorphonuclear leukocyte and T lymphocyte–mediated responses to coccidioidal antigens. In most cases, a mixed inflammatory response is associated with early resolution of the infection and development of a positive delayed hypersensitivity skin test. Progressive disease is associated with weak or absent cellular immunity and skin test anergy. In most infected persons the infection is controlled after mild or inapparent illness. The disease progresses if cell-mediated immunity and consequent macrophage activation do not develop. Such immune deficits may be a result of disease (AIDS) or immunosuppressive therapy but may occur in persons with no other known cellular immune compromise.

Cell-mediated immunity is of prime importance

Progressive disease develops in patients with AIDS or defects in cell-mediated immunity

The central event appears to be the reaction to arthroconidia or to endospores released from ruptured spherules. Arthroconidia can be phagocytosed and killed by polymorphonuclear leukocytes even before an adaptive immune response is mounted. The handling of endospores requires the additional participation of macrophages that do not become maximally effective until activated by T lymphocyte–derived cytokines, particularly those produced by the $T_H1$ subsets. Prior to this, *C. immitis* endospores may be able to impair phagosome–lysosome fusion in the phagocyte.

Endospores must be destroyed by cytokine-activated macrophages

Humoral mechanisms are not known to play any role in immunity. In fact, *C. immitis* is resistant to complement-mediated killing, and levels of complement-fixing antibody are inversely related to the process of disease resolution. Persons with minimal objective indications of tissue involvement (eg, lesions, radiographs) have strong T-lymphocyte responses to *C. immitis* antigens and little if any detectable antibody. Those with disseminated disease and absent cellular immunity have high titers of antibody. Thus, the levels of antibody indicate the extent of antigenic stimulation with no known contribution to resolution of the infection.

Antibody production is inversely related to disease progress

# COCCIDIOIDOMYCOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

More than one half of those infected with *C. immitis* suffer no symptoms, or the disease is so mild that it cannot be recalled when skin test conversion is discovered. Others develop malaise, cough, chest pain, fever, and arthralgia 1 to 3 weeks after infection. This disease, which lasts 2 to 6 weeks, is known as **valley fever** by the local populations in the United States. Objective findings are few. The chest x-ray is usually clear or shows only hilar adenopathy. Erythema nodosum may develop midway through the course, particularly in women. In most cases, resolution is spontaneous but only after considerable discomfort and loss of productivity. In more than 90% of cases, there are no pulmonary residua. A small number of cases progress to a chronic pulmonary form characterized by cavity formation and a slow relapsing course that extends over years. Less than 1% of all primary infections disseminate to foci outside the lung.

Valley fever is usually asymptomatic and self-limiting

Erythema nodosum is common in women

Chronic and disseminated disease less than 1%

Disseminated disease is more common in men; in dark-skinned races, particularly Filipinos; and in AIDS patients and other immunosuppressed persons. Evidence of extrapulmonary infection almost always appears in the first year after infection. The most common sites are bones, joints, skin, and meninges. Coccidioidal meningitis develops slowly with gradually increasing headache, fever, neck stiffness, and other signs of meningeal irritation. The CSF findings are similar to those in tuberculosis and other fungal causes of meningitis, such as *C. neoformans*. Mononuclear cells predominate in the cell count, but substantial numbers of neutrophils are often present. If untreated, the disease is slowly progressive and fatal.

Racial orientation and immune status are risk factors for dissemination

Meningitis is chronic sign

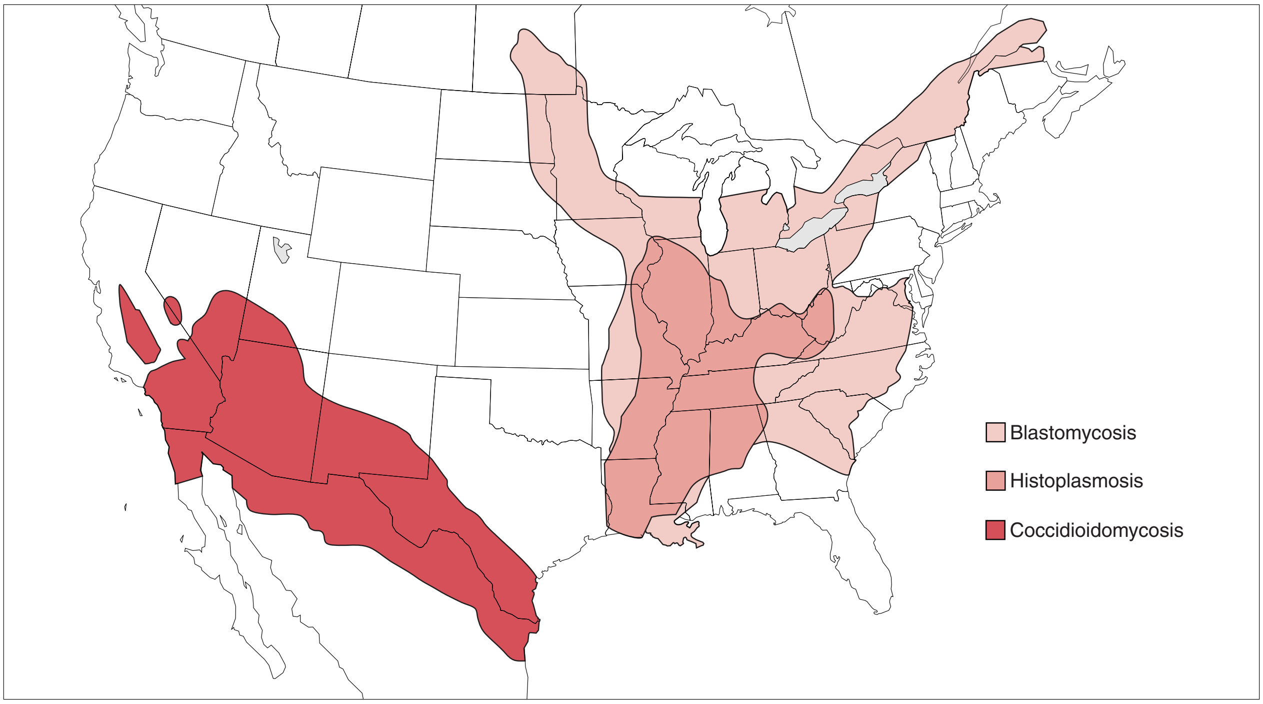


FIGURE 49–7
Geographic distribution of systemic fungal infections in the United States.

## DIAGNOSIS

Direct examination for spherules is diagnostic

With enough persistence, direct examinations are usually rewarding. The thick-walled spherules are so large and characteristic (see Fig 49–4A and C) that they are difficult to miss in a KOH preparation or biopsy section. Skin and visceral lesions are most likely to demonstrate spherules; CSF is least likely. Spherules released into expectorated sputum are often small (10–15 mm) and immature without well-developed endospores. Spherules stain well in histologic sections with either H&E or special fungal stains.

Culture from CSF may be difficult

Substantial risk of laboratory infection with arthroconidia

Culture of *C. immitis* from sputum, visceral lesions, or skin lesions is not difficult, but must be undertaken only by those with experience and proper biohazard protection. Cultures of CSF are positive in less than half the cases of meningitis. Laboratories must be warned of the possibility of coccidioidomycosis to ensure diagnosis and avoid inadvertent laboratory infection. The latter is particularly significant outside the endemic areas, where routine precautions may not be in place. Identification requires observation of typical arthroconidia and demonstration of mycelial antigens using the exoantigen test or a gene probe.

Coccidioidin skin test remains positive for life

Precipitating IgM indicates acute infection

IgG detected by complement fixation quantitates disease

Skin and serologic tests are particularly useful in diagnosis and management of coccidioidomycosis. The coccidioidin skin test usually becomes positive 1 to 4 weeks after the onset of symptoms of primary infection and remains so for life. Although this skin test is generally believed to be useful in clinical diagnosis and management, it is currently not commercially available. Disseminated disease is frequently associated with anergy. One half to three quarters of patients with primary infection develop serum IgM precipitating antibody in the first 3 weeks of illness. These conditions persist for 2 to 4 months. IgG antibodies detected by complement fixation tests appear somewhat later in symptomatic infections. The amount and duration depend on the extent of disease. Antibodies disappear with resolution and persist with continuing infection. The height of the complement fixation titer is a measure of the extent of disease. The presence of complement-fixing antibody in the CSF is also important in the diagnosis of coccidioidal meningitis, because cultures are frequently negative. Precipitating and complement-fixing antibodies may be detected by classic methods or by more recently developed immunoassay procedures.

## TREATMENT

Primary coccidioidomycosis is self-limiting, and no antifungal therapy is indicated. Progressive pulmonary disease and disseminated disease require the use of antifungal agents, usually amphotericin B. Ketoconazole has proved effective, but relapses are common. Fluconazole and itraconazole are also active against *C. immitis*. With the exception of fluconazole, none of these agents have significant penetration into the CNS. Amphotericin B is commonly given directly into the CSF for the treatment of *C. immitis* meningitis.

Amphotericin B in progressive disease

Azoles also active

# *PARACOCCIDIOIDES BRASILIENSIS*

*Paracoccidioides brasiliensis* is the cause of paracoccidioidomycosis (South American blastomycosis), a disease limited to tropical and subtropical areas of Central and South America. The organism is a dimorphic fungus, the most noteworthy feature of which is the production of multiple blastoconidia from the same cell. Characteristic 5- to 40-$\mu$m cells covered with budding blastoconidia may be seen in tissue or in yeast-phase growth at 37°C. The disease manifests primarily as chronic mucocutaneous or cutaneous ulcers. The ulcers spread slowly and develop a granulomatous mulberry-like base. Regional lymph nodes, reticuloendothelial organs, and the lungs may also be involved.

Yeast with multiple blastoconidia are seen in ulcerative lesions

Little is known of the pathogenesis of the disease, although the route of infection is believed to be inhalation. Progression in experimental animals is associated with depressed T lymphocyte-mediated immune responses. The disease has a striking predilection for men, despite skin test evidence that subclinical cases occur at the same rate in both sexes. This may be related to the experimental observation that estrogens but not androgens inhibit conversion of mold-phase conidia to the yeast phase. Treatment is with sulfonamides, amphotericin B, and, more recently, the azole compounds.

Disease has a strong predilection for men

## ADDITIONAL READING

Hogan LH, Klein BS, Levitz SM. Virulence factors of medically important fungi. *Clin Microbiol Rev* 1996;9:469–488. This review addresses aspects of pathogenesis and immunity completely but avoids excessive detail.

Practice guidelines for the management of systemic mycoses: The following four papers are were written by expert Mycoses Study Groups commissioned by the National Institute of Allergy and Infectious Diseases and the Infectious Diseases Society of America to define the management of cryptococcosis, histoplasmosis, blastomycosis, and coccidioidomycosis. Each begins with a concise summary of the disease and concludes with specific recommendations. They are definitive clinical summaries of each disease.

Chapman SW, Bradsher RW Jr, Campbell GD, Pappas PG, Kauffman CA. Practice guidelines for the management of patients with blastomycosis. *Clin Infect Dis* 2000;30: 679–683.

Galgiani JN, Ampel NM, Catanzaro A, Johnson RH, Stevens DA, Williams PL. Practice guideline for the treatment of coccidioidomycosis. *Clin Infect Dis* 2000;30:658–661.

Saag MS, Graybill RJ, Larsen RA, Pappas PG, Perfect JR, Powderly WG, Sobel JD, Dismukes WE. Practice guidelines for the management of cryptococcal disease. *Clin Infect Dis* 2000;30:710–718.

Smith CE, Saito MT, Simons SA. Pattern of 39,500 serologic tests in coccidioidomycosis. *JAMA* 1956;160:546–552. This study is the basis for the unique application of serologic tests to the diagnosis and prognosis of coccidioidomycosis.

Wheat J, Sarosi G, McKinsey, Hamill R, Bradsher R, Johnson P, Loyd J, Kauffman C. Practice guidelines for the management of patients with histoplasmosis. *Clin Infect Dis* 2000;30:688–695.

CHAPTER 50

# *Pneumocystis carinii*

KENNETH J. RYAN

*Pneumocystis carinii* is the cause of a lethal pneumonia of immunocompromised persons, particularly those with AIDS. Its fungal nature is deduced from genomic studies; the organism has not been grown in culture.

## *Pneumocystis carinii*

To date, it has not been possible to cultivate *P. carinii*. Our knowledge of its nature rests on morphologic observations and the study of organisms purified from infected lungs. Until recently, *P. carinii* was believed to be a protozoan, and its "life cycle" was deduced from static images seen in infected tissues. The observed stages include a delicate 5- to 8-$\mu$m cystic structure within which elliptical subunits grow and repeat the cycle on rupture of the cyst. In parasitic parlance, these were called trophozoites, precysts, and cysts. The corresponding mycologic terms based on the same observations are spore, sporocyte, and spore case (Fig 50–1).

Life cycle is deduced from static images

Elliptical spores in sporocyte form spore case

The spherical sporocyte (precyst) is bounded by a cell wall and cytoplasmic membrane that enclose a nucleus and several mitochondria. As the precyst matures, the nuclei divide to form the eight spores (trophozoites) within the original structure to form the spore case (cyst). The spores have an eccentric nucleus, a nucleolus, and a single mitochondrion in the cytoplasm.

Eight spores each have nucleus and mitochondria

The confusion about the classification of *Pneumocystis* is understandable. The shape, nucleus, and reticular cytoplasm of the **spores** resemble protozoa, as does their aggregation into the cyst-like **spore case**. The cell wall lacks the rigidity typical of other fungi; however, biochemical elements of the fungal cell wall appear to be present. These include glucan and N-acetylglucosamine, the major subunit of chitin. The dominant sterol of the *P. carinii* cytoplasmic membrane is cholesterol, rather than the ergosterol characteristic of fungi. Other biochemical analyses, however, support the fungal nature such as the presence of elements of protein synthesis (elongation factor 3) that is unique to fungi. The fungal classification of *P. carinii* is most strongly supported by sequence analysis of the genes coding for ribosomal RNA, mitochondrial proteins, and major enzymes. These sequences show the closest homology with fungi and molecular phylogenic analysis, which places *Pneumocystis* near the ascomycetes.

Spores morphologically resemble protozoa

Cell wall is thin, but glucan and chitin elements are present

rRNA and mitochondrial gene sequences are homologous with fungi

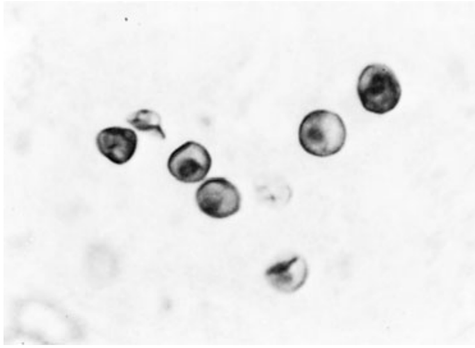

FIGURE 50–1
Sporocytes (cysts) of *Pneumocystis carinii* with developing spore nuclei.

# PNEUMOCYSTOSIS

**CLINICAL CAPSULE**

*Pneumocystis* pneumonia is insidious, beginning with mild fever or malaise in individuals whose immune system is compromised. Signs referable to the lung come later with nonproductive cough and shortness of breath. Radiographs reveal symmetrical alveolar pulmonary infiltrates, which spread from the hili. Progressive cyanosis, hypoxia, and asphyxia can lead to death in a 3- to 4-week period.

## EPIDEMIOLOGY

Worldwide distribution in humans and animals

Antibodies are common

Airborne transmission is probable

Pulmonary infection with *P. carinii* occurs worldwide in humans and a broad spectrum of animal life. Exposure must be common; specific antibodies are present in nearly all children by the age of 4. The reservoir and mode of transmission remain unknown, but the view that the majority of *Pneumocystis carinii* pneumonia (PCP) cases represent reactivation of latent infection is no longer held. *P. carinii* is not found in the respiratory tract of asymptomatic persons, even among HIV-infected individuals, and the strains involved in second and third episodes are frequently antigenically different. Animal studies have shown that airborne transmission is possible, and the circumstances of hospital outbreaks point to active cases as a probable source.

PCP is a complication of immunodeficient states

AIDS patients are at high risk

Before the acquired immunodeficiency syndrome (AIDS) pandemic, PCP occurred sporadically among infants with congenital immunodeficiencies and in older children and adults as a complication of immunosuppressive therapy. Now AIDS has become the most common predisposing condition in the United States, and PCP is often the presenting manifestation of AIDS. In fact, prior to the development of effective chemoprophylactic regimens (see Treatment and Prevention), it was present in approximately half of all AIDS patients at the time of initial diagnosis. Eventually, most AIDS patients develop one or more bouts of PCP, often in conjunction with another opportunistic infection.

## PATHOGENESIS

Low CD4 counts increase the risk in AIDS

*P. carinii* is an organism of low virulence that seldom produces disease in a host with normal T-lymphocyte function. In experimental animals, progressive infection can be initiated with starvation or corticosteroid administration, and in AIDS patients the risk of developing pneumocystosis increases dramatically once the CD4+ T lymphocyte count has fallen to 200 cells/mm$^3$ or below. Concurrent viral, bacterial, fungal, and protozoan infections are found frequently in human cases, suggesting that *P. carinii* may require the presence of another microbial agent for its multiplication.

Little is known about the early stages of disease. A **major surface glycoprotein** (MSG) abundant on the surface of *P. carinii* may act as an attachment ligand to several

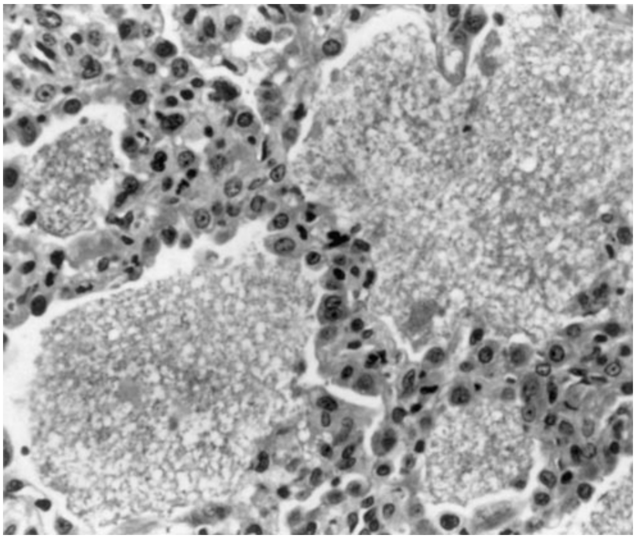

FIGURE 50–2
Lung biopsy specimen from *Pneumocystis carinii*–infected person, showing "foamy" contents of alveoli. (*Reproduced with permission from Connor DH, Chandler FW, Schwartz DA, Manz HJ, Lack EE (eds).* Pathology of Infectious Disease, *Volume II. Stamford, CT: Appleton & Lange, 1997.*)

host proteins, including fibronectin, vitronectin and surfactant proteins. MSG undergoes antigenic variation, which could aid in its persistence in human hosts. Histologically, PCP is characterized by alveoli filled with desquamated alveolar cells, monocytes, organisms, and fluid, producing a distinctive foamy, honeycombed appearance (Fig 50–2); hyaline membranes may be present, and round cell infiltrates may be visible in the septa. It has been suggested that *P. carinii* maintains an extracellular existence within alveoli obtaining essential nutrients from the alveolar fluid and lining cells.

MSG attaches to pneumocytes

Alveoli are filled with foamy exudate

## IMMUNITY

The nature of the immunodeficiencies in patients with pneumocystosis points to the primacy of cell-mediated immunity (CMI) in resolution of infection with *P. carinii.* Alveolar macrophages are the first line of defense, with activated macrophages and CD4+ lymphocytes playing essential roles in the resolution of the infection. Activated macrophages release several cytotoxic factors, including $O_2$-derived radicals, reactive nitrogen intermediates, and cytokines (tumor necrosis factor-$\alpha$, interleukin-2).

Activated macrophages and cytokines mediate CMI

Specific antibody responses to the MSG and other antigens appear in the course of pneumocystosis. A significant role for humoral immunity is suggested by the ability of MSG antibody to protect against experimental PCP in animals.

Antibody plays a role in protection

# PNEUMOCYSTOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

In the immunocompromised host, the disease presents as a progressive, diffuse pneumonitis. Illness may begin after discontinuation or a decrease in the dose of corticosteroids or, in the case of acute lymphatic leukemia, during a period of remission. In infants and AIDS patients, onset is typically insidious, and the clinical course is 3 to 4 weeks in duration. Fever is mild or absent. In older individuals and patients who have previously been on high doses of corticosteroids, the onset is more abrupt, and the course is both febrile (38–40°C) and abbreviated. In both populations, the cardinal manifestations are progressive dyspnea and tachypnea; cyanosis and hypoxia eventually supervene. A nonproductive cough is present in 50% of all patients. Clinical signs of pneumonia are usually absent, despite the presence of infiltrates on x-ray. These infiltrates are alveolar in

Diffuse pneumonitis with insidious onset

Nonproductive cough, dyspnea, and cyanosis develop later

Alveolar infiltrates spread out from the hili character and spread out symmetrically from the hili, eventually affecting most of the lung. Occasionally, unilateral infiltrates, coin lesions, lobar infiltrates, cavitary lesions, or spontaneous pneumothoraces are observed. Pleural effusions are uncommon. Clinical and radiographic abnormalities are generally accompanied by a decrease in arterial oxygen saturation, diffusion capacity of the lung, and vital capacity. Death occurs by progressive asphyxia.

Extrapulmonary lesions are seen in AIDS

Lesions outside the lung were rarely seen prior to the AIDS epidemic but are now seen with some regularity. The sites most often involved are lymph nodes, bone marrow, spleen, liver, eyes, thyroid, adrenal glands, gastrointestinal tract, and kidneys. The extrapulmonary clinical manifestations range from incidental autopsy findings to progressive multisystem disease.

## DIAGNOSIS

Diagnostic yield from sputum is low

BAL is the best of the invasive procedures

Definite diagnosis depends on finding organisms of typical morphology in appropriate specimens. Because the pathologic process is alveolar rather than bronchial, the organisms are not readily seen in expectorated specimens such as sputum. The diagnostic yield is much better from specimens obtained by more invasive procedures. Of these, bronchoalveolar lavage (BAL) gives the best results with the least morbidity. Percutaneous needle aspiration of the lung, transbronchial biopsy, and open lung biopsy, although somewhat more sensitive techniques, are accompanied by more complications, including pneumothorax and hemothorax.

Silver and other stains readily demonstrate *P. carinii*

DFA is sensitive

*P. carinii* can be demonstrated by a wide variety of staining procedures. The standard stain is methenamine silver (Fig 50–3), but a direct fluorescent antibody (DFA) method, if available, is slightly more sensitive. Laboratories often perform a rapid stain (Wright, Giemsa, Papanicolaou) first and confirm by methenamine silver or DFA later. Methods developed for detection of *Pneumocystis* DNA in BAL and other specimens by polymerase chain reaction may soon be practical for clinical laboratories.

## TREATMENT AND PREVENTION

TMP-SMX is treatment of choice

Treatment is extended in AIDS

The fixed combination of trimethoprim and sulfamethoxazole (TMP-SMX) is the treatment of choice for all forms of pneumocystosis. It is administered orally or intravenously for 14 to 21 days. Patients with AIDS receive the longer course because they start with a higher organism burden, respond more slowly, and suffer relapse more often. Unfortunately, AIDS patients have a high incidence of adverse effects to TMP-SMX, particularly the sulfonamide component. This requires the use of other antimicrobics (eg, clindamycin, primaquine, dapsone) alone or in combination with TMP. In hospitalized patients, two parenteral drugs, pentamidine and trimetrexate, are the major alternatives to TMP-SMX.

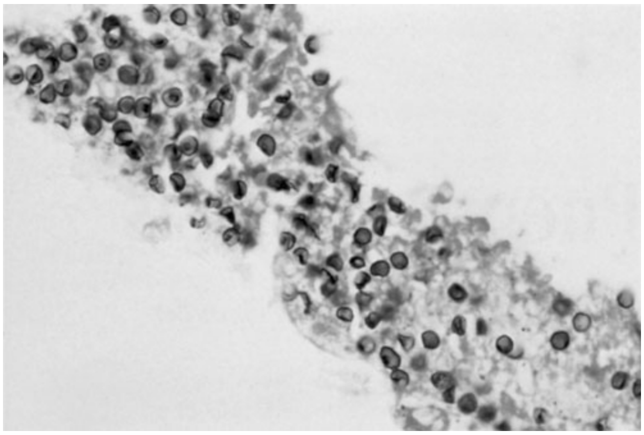

FIGURE 50–3
Multiple spherical and collapsed sporocytes (cysts) of *Pneumocystis carinii* stained by methenamine silver. Note the comma-shaped developing spores. (*Reproduced with permission from Connor DH, Chandler FW, Schwartz DA, Manz HJ, Lack EE (eds).* Pathology of Infectious Disease, *Volume II. Stamford, CT: Appleton & Lange, 1997.*)

Low-dose administration of TMP-SMX has been shown to significantly decrease the incidence of *P. carinii* pneumonia in high-risk patients and prevents relapse in AIDS patients. This chemoprophylaxis is indicated for patients who have CD4+ lymphocyte counts below 200/mm$^3$, unexplained fever, or a previous episode of PCP. Once begun, chemoprophylaxis is continued for life.

Chemoprophylaxis prevents PCP in high-risk groups

## ADDITIONAL READING

Kovacs JA, Gill VJ, Meshnick S, Masur H. New insights into transmission, diagnosis, and drug treatment of *Pneumocystis carinii* pneumonia. *JAMA* 2001; 286:2450–2460. An excellent, concise review, which also points out that mutants may be emerging that are resistant to sulfa drugs and atovaquone.

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PART VIII

# PARASITES

## CHAPTER 56

Tissue Nematodes

## CHAPTER 57

Cestodes

## CHAPTER 58

Trematodes

CHAPTER 51

# Introduction to Pathogenic Parasites: Pathogenesis and Chemotherapy of Parasitic Diseases

JAMES J. PLORDE

This chapter provides an overview of parasitic diseases and of antiparasitic therapy. The student may find it valuable to reread it after studying the subsequent chapters in this section.

## DEFINITION

Within the context of this section of the book, the term **parasite** refers to organisms belonging to one or two major taxonomic groups: protozoa and helminths. Protozoa are microscopic, single-celled eukaryotes superficially resembling yeasts in both size and simplicity. Helminths, in contrast, are macroscopic, multicellular worms possessing differentiated tissues and complex organ systems; they vary in length from a meter to less than a millimeter. The majority of both protozoa and helminths are free-living, play a significant role in the ecology of the planet, and seldom inconvenience the human race. The less common disease-producing species are typically obligate parasites, dependent on vertebrate hosts, arthropod hosts, or both for their survival. When their level of adaptation to a host is high, their presence typically produces little or no injury. Less complete adaptation leads to a more serious disturbance of the host and, occasionally, to death of both host and parasite.

Eukaryotic single-celled protozoa and multicellular macroscopic helminths

Most are free living

Disease-producing species usually obligate parasites

## SIGNIFICANCE OF HUMAN PARASITIC INFECTIONS

The relative infrequency of parasitic infections in the temperate, highly sanitated societies of the industrialized world has sometimes led to the parochial view that knowledge of parasitology has little relevance for physicians practicing in these areas. The continuing presence of parasitic disease among the impoverished, immunocompromised, sexually active, and peripatetic segments of industrialized populations, however, means that most physicians will regularly encounter those pathogens. Parasitic diseases remain among the major causes of human misery and death in the world today and, as such, are important obstacles to the development of the economically less favored nations (Table 51–1).

Major cause of disease and death worldwide

Moreover, a number of recent medical, socioeconomic, and political phenomena have combined to produce a dramatic recrudescence of several parasitic diseases with important consequences to both the United States and the developing world.

Resistance of malarial parasites to chemotherapeutics

Resistance of insect vectors to insecticides

Recent increases in imported malaria

Currently, 2.5 billion people live in malarious areas, and of these, approximately 500 million are infected at any given time. Between 1 and 3 million people, predominately children, die of malaria each year. *Plasmodium falciparum,* the most deadly of the malarial organisms, has developed resistance to several categories of antimalarial agents, and resistant strains are now found throughout Southeast Asia, parts of the Indian subcontinent, southeast China, large areas of tropical America, and tropical Africa. Growing resistance of the mosquito vector of malaria to the less toxic and less expensive insecticides has resulted in a cutback of many malaria control programs. In countries such as India, Pakistan, and Sri Lanka, where eradication efforts had previously interrupted parasite transmission, the disease incidence has increased 100-fold in recent years. In tropical Africa, the intensity of transmission defies current control measures. Of direct interest to

TABLE 51–1

**Prevalence of Parasitic Infections**

| Disease | Estimated Population Affected |
|---|---|
| **Amebiasis** | 10% of world population |
| Annual deaths | 40–110 thousand |
| **Giardiasis** | 200 million |
| **Malaria** | 400–490 million |
| Population at risk | 2.5 billion |
| Annual deaths | 2–3 million |
| **Leishmaniasis** | 12 million |
| **African trypanosomiasis** | |
| Population at risk | 50 million |
| New cases per year | 100,000 |
| Annual deaths | 5000 |
| **American trypanosomiasis** | 24 million |
| Population at risk | 65 million |
| New cases per year | 60,000 |
| **Schistosomiasis** | 200 million |
| Population at risk | 600 million |
| Annual deaths | 0.5–1.0 million |
| **Clonorchis** and **opisthorchiasis** | 13.5 million |
| **Paragonimiasis** | 2.1 million |
| **Fasciolopsiasis** | 10 million |
| **Filiariasis** | 128 million |
| **Onchocerciasis** | 18 million |
| **Dracunculiasis** | <100,000 |
| **Ascariasis** | 1.3 billion |
| **Hookworm** | 1.3 billion |
| **Trichuriasis** | 0.9 billion |
| **Strongyloidiasis** | 35 million |
| **Enterobius vermicularis** | 400 million |
| **Cestodiasis** | 65 million |

American physicians is the spillover of this phenomenon to the United States. Presently, approximately 1000 cases of imported malaria are reported annually.

*Entamoeba* spp. are intestinal protozoa that infect 10% of the world's population, including 2 to 3% in the United States. The majority of individuals are infected with the noninvasive *E. dispar.* The invasive *E. histolytica* produces amebiasis, a disease characterized by intestinal ulcers and liver abscesses. It is more commonly seen in the poorly sanitated areas of the world, but occurs in the United States as well, particularly in institutions for the mentally retarded and among migrant workers and some male homosexuals.

Amebic infections in 10% of world population

In the poor, rural areas of Latin America, *Trypanosoma cruzi* infects an estimated 16 million individuals annually, leaving many with the characteristic heart and gastrointestinal lesions of Chagas' disease. In Africa, from the Sahara Desert in the north to the Kalahari in the south, a related organism, *Trypanosoma brucei,* causes one of the most lethal of human infections, sleeping sickness. Animal strains of this same organism limit food supplies by making the raising of cattle economically unfeasible.

Trypanosomiasis produces disease and limits food supplies

Leishmaniasis, a disease produced by another intracellular protozoan, is found in parts of Europe, Asia, Africa, and Latin America. Clinical manifestations range from a self-limiting skin ulcer, known as oriental sore, through the mutilating mucocutaneous infection of espundia, to a highly lethal infection of the reticuloendothelial system (kala azar).

Leishmaniasis can cause cutaneous or disseminated disease

In 1947 Stoll, in an article entitled "This Wormy World," estimated that between the tropics of Cancer and Capricorn there were many more intestinal worm infections than people. The prevalence was judged to be far lower in temperate climates. Warren, however, recently estimated that 27% of the American population harbored worms. The most serious of the helminthic diseases, schistosomiasis, affects an estimated 200 million individuals in Africa, Asia, and the Americas. Individuals with heavy worm levels develop bladder, intestinal, and liver disease, which may ultimately result in death. Unfortunately, the disease is frequently spread as a consequence of rural development schemes. Irrigation projects in Egypt, the Sudan, Ghana, and Nigeria have significantly increased the incidence of the disease in these areas, often mitigating the economic gains of the development program itself.

Parasitic worm infections prevalent, may be spread by irrigation projects

Two closely related filarial worms, *Wuchereria bancrofti* and *Brugia malayi,* which are endemic in Asia and Africa, interfere with the flow of lymph and can produce grotesque swellings of the legs, arms, and genitals. Another filaria produces onchocerciasis (river blindness) in millions of Africans and Americans, leaving thousands blind.

Filariasis produces swellings

Toxoplasmosis, giardiasis, trichomoniasis, and pinworm infections are four cosmopolitan parasitic infections well known to American physicians. The first, a protozoan infection of cats, infects possibly one third of the world's human population. Although it is usually asymptomatic, infection acquired in utero may result in abortion, stillbirth, prematurity, or severe neurologic defects in the newborn. Asymptomatic infection acquired either before or after birth may subsequently produce visual impairment. Immunosuppressive therapy may reactivate latent infections, producing severe encephalitis.

Multiple parasitic diseases common in the United States

## BIOLOGY, MORPHOLOGY, AND CLASSIFICATION

### Protozoa

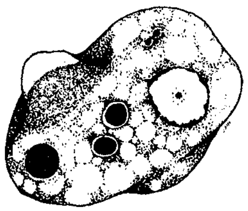
#### *Morphology*

Protozoa range in size from 2 to more than 100 $\mu$m. Their protoplasm consists of a true membrane-bound nucleus and cytoplasm. The former contains clumped or dispersed chromatin and a central nucleolus or **karyosome.** The shape, size, and distribution of these structures are useful in distinguishing protozoan species from one another.

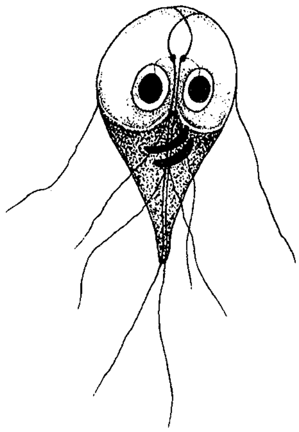
The cytoplasm is frequently divided into an inner endoplasm and a thin outer ectoplasm. The granular **endoplasm** is concerned with nutrition and often contains food reserves, contractile vacuoles, and undigested particulate matter. The **ectoplasm** is organized into specialized organelles of locomotion. In some species, these organelles appear as blunt, dynamic extrusions known as pseudopods. In others, highly structured thread-like cilia or flagella arise from intracytoplasmic basal granules. Flagella are longer and less numerous than cilia and possess a structure and a mode of action distinct from those seen in prokaryotic organisms.

Endoplasm contains nutrients

Ectoplasm has organelles of locomotion



Prototypic rhizopod



Prototypic flagellate
(*Giardia*)

10 µm

TABLE 51–2

**Classes of Protozoa**

| Class | Organelles of Locomotion | Method of Reproduction |
|---|---|---|
| Rhizopods (amebas) | Pseudopods | Binary fission |
| Ciliates | Cilia | Binary fission |
| Flagellates | Flagella | Binary fission |
| Sporozoa | None | Schizogony/sporogony |

## *Classification*

Mode of reproduction and type of locomotive organelle are used to divide the protozoa into four major classes (Table 51–2). Although most **rhizopods** (amebas) are free-living, several are found as commensal inhabitants of the intestinal tract in humans. One of these organisms, *E. histolytica,* may invade tissue and produce disease. Occasionally, free-living amebas may gain access to the body and initiate illness. The majority of **ciliates** are free-living and seldom parasitize humans. **Flagellates** of the genera *Trypanosoma* and *Leishmania* are capable of invading the blood and tissues of humans, where they produce severe chronic illness. Others, such as *Trichomonas vaginalis* and *Giardia lamblia,* inhabit the urogenital and gastrointestinal tracts and initiate disease characterized by mild to moderate morbidity but no mortality. **Sporozoan** organisms, in contrast, produce two of the most potentially lethal diseases of humans, malaria and toxoplasmosis.

Most amebas are either free-living or commensal

Protozoa are facultative anaerobes

Nutrients engulfed by phagocytosis or pinocytosis

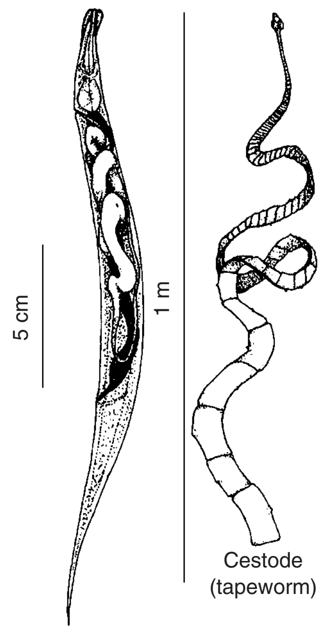
Many protozoa form resistant cysts as survival form

Reproduction usually by binary fission

## *Physiology*

Most parasitic protozoa are facultative anaerobes. They are heterotrophic and must assimilate organic nutrients. This assimilation is accomplished by engulfing soluble or particulate matter in digestive vacuoles, processes termed **pinocytosis** and **phagocytosis,** respectively. In some species, food is ingested at a definite site, the peristome or cytostome. Food may be retained in special intracellular reserves, or vacuoles. Undigested particles and wastes are extruded at the cell surface by mechanisms that are the reverse of those used in ingestion.

Survival is ensured by highly developed protective and reproductive techniques. Many protozoa, when exposed to an unfavorable milieu, become less active metabolically and secrete a cyst wall capable of protecting the organism from physical and chemical conditions that would otherwise be lethal. In this form, the parasite is better equipped to survive passage from host to host in the external environment. Immunoevasive mechanisms described later (see Immunity) contribute to survival within the host. Reproduction is accomplished primarily by simple binary fission. In one class of protozoa, the Sporozoa, a cycle of multiple fission (schizogony) alternates with a period of sexual reproduction (sporogony).




Cestode
(tapeworm)

Prototypic nematode
(roundworm)

# Helminths

## *Morphology and Classification*

Worms are elongated, bilaterally symmetric animals that vary in length from less than a millimeter to a meter or more. The body wall is covered with a tough acellular **cuticle,** which may be smooth or possess ridges, spines, and tubercles. At the anterior end, there are often suckers, hooks, teeth, or plates used for the purpose of attachment. All helminths have differentiated organs. Primitive nervous and excretory systems and a highly developed reproductive system are characteristic of the entire group. Some have alimentary tracts; none possess a circulatory system. The common helminthic parasites of humans can be placed in one of three classes on the basis of body and alimentary tract configuration, nature of the reproductive system, and need for more than a single host species for the completion of the life cycle (Table 51–3).

TABLE 51–3

**Classification of Helminthic Parasites of Humans**

| Characteristic | Roundworm (Nematode) | Tapeworm (Cestode) | Fluke (Trematode) |
|---|---|---|---|
| Morphology | Spindle-shaped | Head with segmented body (proglottids) | Leaf-shaped with oral and ventral suckers |
| Sex | Separate sexes | Hermaphroditic | Hermaphroditic[a] |
| Alimentary tract | Tubular | None | Blind |
| Intermediate host | Variable[b] | One[c] | Two[d] |

[a] *Schistosoma* group has separate sexes.

[b] Tissue nematodes have intermediate hosts; intestinal nematodes do not.

[c] *Diphyllobothrium* group has two hosts.

[d] *Schistosoma* group has one host.

**Roundworms,** or nematodes, have a cylindrical fusiform body and a tubular alimentary tract that extends from the mouth at the anterior end to the anus at the posterior end. The sexes are separate, and the male worm is typically smaller than the female. These worms can be divided into those that dwell within the gastrointestinal tract and those that parasitize the blood and tissues of humans. Unlike the latter, those in the gastrointestinal tract generally do not require intermediate hosts.

**Tapeworms,** or cestodes, have flattened, ribbon-shaped bodies. The anterior end, or **scolex,** is armed with suckers and frequently with **hooklets,** which are used for attachment. Immediately behind the head is a neck that generates a chain of reproductive segments, or **proglottids.** Each segment contains both male and female gonads. The worm lacks a digestive tract and presumably absorbs nutrients across its cuticle. One or sometimes two intermediate hosts are required for completion of the life cycle.

Differentiated organs: no circulatory system

Proglottids contain male and female gonads

**Flukes,** or trematodes, are leaf-shaped organisms with blind, branched alimentary tracts. Particulate waste is regurgitated through the mouth. Two suckers, one surrounding the mouth and the second located more distally on the ventral aspect of the body, serve as organs of attachment and locomotion. Most are hermaphroditic and require two intermediate hosts. The blood-dwelling schistosomes, however, are unisexual and require but a single intermediate.

Most require two intermediate hosts

## *Physiology*

Helminthic parasites are nourished by ingestion or absorption of the body fluids, lysed tissue, or intestinal contents of their hosts. Carbohydrates are rapidly metabolized, and the glycogen concentration of the worms is high. Respiration is primarily anaerobic, although larval offspring frequently require oxygen. A large part of the energy requirement is devoted to reproductive needs. The daily output of offspring can be as high as 200,000 for some worms. Typically, helminths are **oviparous** (excrete eggs), but a few species are **viviparous** (give birth to living young). The egg shells of many parasites with an aquatic intermediate host possess a lidded opening, or **operculum,** through which the embryo escapes once the egg reaches water. Whether hatched or freeborn, the resulting larva is morphologically distinct from the mature worm and undergoes a series of changes or molts before it achieves adulthood.

Anaerobic respiration of adult worm

High fertility producing thousands of eggs

Protection from the host's digestive and body fluids is afforded by the tough cuticle and the secretion of enzymes. Some worms, such as the schistosomes, can protect themselves from immunologic attack by the incorporation of host antigens into their cuticles. The life span of the adult helminth is often measured in weeks or months, but some, such as the hookworms, filaria, and flukes, can survive within their hosts for decades.

Mechanical protection from environment and immunologic attack

TABLE 51–4

**Transmission and Distribution of Four Representative Parasites**

| Organism | Infective Form | Mechanism of Spread | Distribution |
|---|---|---|---|
| *Trichomonas vaginalis* | Trophozoite | Direct (venereal) | Worldwide |
| *Entamoeba histolytica* | Cyst/trophozoite | Direct (venereal) | Worldwide |
| | Cyst | Indirect (fecal–oral) | Areas of poor sanitation |
| *Ascaris lumbricoides* | Egg | Indirect (fecal–oral) | Areas of poor sanitation |
| *Plasmodium falciparum* | Sporozoite | *Anopheles* mosquito | Tropical and subtropical areas |

## LIFE CYCLES, TRANSMISSION, AND DISTRIBUTION

### Single-Host Parasites

As is evident from the previous discussion, many parasites require but a single host species for the completion of their life cycles. The method by which the parasite is transmitted from individual to individual within that species is determined in large part by its viability in the external environment and, in the case of helminths, by the conditions required for the maturation of offspring. The mode of transmission, in turn, determines the social, economic, and geographic distribution of the parasite. A few examples are described in Table 51–4.

Transmission by direct sexual contact

The protozoan *T. vaginalis* does not produce protective cyst forms. Although its active, or trophozoite, form is relatively hardy, it can survive only a few hours outside of its normal habitat, the human genital tract. Thus, for all practical purposes, transmission requires the direct genital contact of sexual intercourse. As a result, trichomoniasis is cosmopolitan, occurring wherever human hosts engage in sexual activity with multiple partners.

Fecal–oral transmission common for less fragile intestinal parasites

Another protozoan, *E. histolytica,* inhabits the human gut and produces hardy **cysts** that are passed in the stool. Transmission occurs when another individual ingests these cysts. Like *T. vaginalis,* the organism can be passed by direct physical contact, in this case by oral–anal sexual activity. This mode of transmission, in fact, accounts for the high incidence of amebic infections in male homosexuals. Unlike *T. vaginalis,* however, the cysts can survive prolonged periods in the external environment, where they may eventually contaminate food or drinking water. Thus, in environments such as mental institutions, where the level of personal hygiene is low, or in populations in which methods for the sanitary disposal of human wastes are not available, amebiasis is common.

Some require infectivity development in soil

The intestinal helminth *Ascaris lumbricoides* illustrates still another transmission pattern. In this infection, highly resistant eggs are passed in the human stool. Unlike the situation with *E. histolytica* described previously, the eggs are not immediately infective but must incubate in soil under certain conditions of temperature and humidity before they are fully embryonated and infectious. As a result, this parasite cannot be transmitted directly from host to host. The organism spreads only when indiscriminate human defecation results in deposition of eggs on soil and subsequent exposure of that soil to the climatic conditions required for embryonation of the eggs. For this reason, *Ascaris* infections are most prevalent in poorly sanitated areas of the tropics and subtropics.

### Multiple-Host Parasites

Multiple hosts may be involved in life cycle

Definitive host and one or more intermediate hosts

A few protozoa and many helminths require two or more host species in their life cycle. To avoid confusion, it is customary to refer to the species in which the parasite reproduces sexually as the **definitive host** and that in which asexual reproduction or larval development takes place as the **intermediate host.** When there is more than one intermediate, they are known simply as the first and second intermediate hosts. In some cases, such as that of *Taenia saginata,* the beef tapeworm, both host species are vertebrates; humans serve as the definitive host and cattle as the intermediate. Among parasites that inhabit the blood and tissues of humans, it is more common for a blood-feeding arthropod to serve as a second host and as the transmitting vector. An example is malaria, in which the causative plasmodium is transmitted from person to person by the bite of an infected female mosquito of the genus *Anopheles*. In this particular instance, sexual reproduction occurs in the mosquito, making it the definitive host and relegating the human host to the role of a mere intermediate.

The distribution of parasites requiring a nonhuman host is limited to the ecologic niche occupied by this second host. Thus, the areas in which malaria is endemic are restricted by the distribution of *Anopheles* mosquitos. The area of disease distribution is, in fact, generally smaller than that of the nonhuman host, because conditions favoring parasite transmission may also differ. For example, both the abundance of *Anopheles* and the speed with which the malarial parasite completes its development within them are directly related to the ambient temperature and humidity. Among temperate zone *Anopheles,* the number of infected mosquitoes may be insufficient to sustain parasite transmission. In tropical areas, transmission is more likely to be constant and intense. In another more obvious example, infections with *T. saginata* are found only in areas where cattle are raised for human consumption and, within those areas, only where indiscriminate human defecation and the ingestion of raw or undercooked beef are common.

Distribution of nonhuman hosts influences disease occurrence

## IMMUNITY

The large size, complex structure, varied metabolic activity, and synthetic prowess of most parasites provide their human host with an intense antigenic challenge. Generally, the resulting immunologic response is vigorous, but its role in modulating the parasitic invasion differs significantly from that in viral and bacterial infections. It is apparent from the chronic course and frequent recurrences typical of many parasitic diseases that acquired resistance is often absent. When present, it is generally incomplete, serving to moderate the intensity of the infection and its associated clinical manifestations rather than to destroy or expel the causative pathogen. In fact, clinical recovery and resistance to reinfection in some parasitoses require the persistence of viable organisms at low concentration within the body of the host **(premunition).** Complete sterilizing immunity with prolonged resistance to reinfection is exceptional.

Immune response to parasites vigorous but often relatively ineffective

This pusillanimous response does not result from any dearth of immunologic mechanisms available to the host. All those generally exercised against the more primitive microorganisms, including antibodies, cytotoxic T lymphocytes, activated macrophages, natural killer cells, antibody-dependent cell-mediated toxicity, lymphokines, and complement activation, have been shown to play a part in moderating parasitic infection. In worm infections, some of these mechanisms find unique implementation. On invasion of tissue, helminths stimulate the production of IgE, the Fc portion of which binds to mast cells and basophils. Interaction of the antibody with parasitic antigen triggers the release of histamine and other mediators from the attached cells. These may injure the worm directly or, by increasing vascular permeability and stimulating the release of chemotactic factors, may lead to the accumulation of other cells and IgG antibodies capable of initiating antibody-dependent, cell-mediated destruction of the parasite. The specific killer cell involved is often the eosinophil. These cells attach by their Fc receptor site to IgG antibody-coated parasites and degranulate, releasing a major basic protein that is directly toxic to the worm.

All elements of immune response mobilized

IgE response to worm infections attracts eosinophils

Eosinophils bind to IgG-coated parasite and release toxic protein

The techniques by which parasites have been shown to evade the consequences of the host's specific acquired immunity are numerous. Included among them are seclusion within immunologically protected areas of the body, continual alteration of surface antigens, and active suppression of the host's effector mechanisms. A number of protozoa are shielded from humoral defenses by virtue of their intracellular location. Some have even found ways to avoid or survive the normally lethal environment of the phagolysosome of the macrophage. *T. cruzi,* for example, lyses the phagosomal membrane, providing escape into the cytoplasm, whereas *Toxoplasma gondii* inhibits fusion of the phagosome with lysosomes. *Leishmania* species, capable of neither of these feats, are resistant to the action of lysosomal enzymes and survive in the phagolysosomes.

Some intracellular protozoa avoid phagolysosome destruction

*Toxoplasma,* cestode larvae, and *Trichinella spiralis* armor themselves against immunologic attack by encysting within the tissue of the host. The gut lumen is, perhaps, Encysted and intestinal parasites relatively inaccessible to host defenses

the largest immunologic sanctuary within the body, because, unless the integrity of the intestinal mucosa is breached by injury or inflammation, this barrier protects lumen-dwelling parasites from most of the effective humoral and cellular immune mechanisms of the host, allowing almost unfettered growth and multiplication.

Antigenic shifts occur with developmental changes in parasite

Trypanosomal antigenic variation outpaces immunologic response

Antigenic glycoprotein variants of trypanosomes selected from preexisting genetic repertoire

Most immune effector mechanisms are directed against the surface antigens of the parasite, and alteration of these antigens may blunt the immunologic attack. Many parasites undergo developmental changes within their hosts that are generally accompanied by alterations in surface antigens. Immune responses directed at an early developmental stage may be totally ineffective against a later stage of the same parasite. Such stage-specific immunity has been demonstrated in malaria, schistosomiasis, and trichinosis, accounting for the seeming paradox of parasite survival in a host resistant to reinfection with the same strain of organism. Even more intriguing is the ability of some parasites to vary the antigenic characteristics of a single developmental stage. The trypanosomes that cause African sleeping sickness circulate in the bloodstream coated with a thick layer of glycoprotein. The development of humoral antibody to this coating results in the elimination of the parasite from the blood. This is followed by successive waves of parasitemia, each associated with a new glycoprotein antigen on the parasite against which the previously produced antibody is ineffective. The parasite is capable of producing more than 100 glycoprotein variants, each encoded by a different structural gene. The expression of individual genes from this large genetic repertoire is controlled by the sequential transfer of a duplicate copy of each gene to an area of the parasite responsible for gene expression.

Antigenic shedding and masking with host antigens

Several protozoan and helminthic pathogens are thought to be capable of neutralizing antibody-mediated attack by shedding and, later, regenerating specific surface antigens. Adult schistosomes, in addition, may immunologically hide from the host by masking themselves with host blood group antigens and immunoglobulins.

Parasites may destroy immunologic mediators

Some parasites cause immune suppression

A number of parasites can destroy or inactivate immunologic mediators. Tapeworm larvae produce anticomplementary chemicals, and *T. cruzi* splits the Fc component of attached antibodies, rendering it incapable of activating complement. Several protozoa, most notably *T. brucei,* the etiologic agent of African sleeping sickness, induce polyclonal B-cell activation leading to the production of nonspecific immunoglobulins and eventual exhaustion of the antibody-producing capacity of the host. This and other protozoa can produce nonspecific suppression of both cellular and humoral effector mechanisms, enhancing the host's susceptibility also to a variety of unrelated secondary infections. Patients with disseminated leishmaniasis display a specific inability to mount a cellular immune response to parasitic antigens in the absence of evidence of generalized immunosuppression.

Cuticle helps resist immune effectors

Finally, the thick, tough cuticle of many adult helminths renders them impervious to immune effector mechanisms designed to deal with the less robust microbes.

## PATHOGENESIS

Most adult helminths do not multiply within host

Disease severity related to worm load

In helminthic infections, humans may serve as the definitive host to the sexually mature adult worms (eg, *Taenia saginata*) or as the intermediate host to the larval stages (eg, *Echinococcus granulosus*). Occasionally, they serve as both the definitive and the intermediate host to the same worm (eg, *Trichinella spiralis, Taenia solium*). Unlike protozoan parasites, most adult helminths are incapable of increasing their numbers within their definitive host. As a result, the severity of clinical illness is related to the total number of worms acquired by the host over time. Most small worm loads are, in fact, asymptomatic and may not require therapy. Many worms are long-lived, however, and repeated infections can result in very high worm loads with subsequent disability. The pathogenesis of both protozoan and helminthic disease is highly variable. The fish tapeworm *Diphyllobothrium latum* competes with the host for nutrients. The protozoan *Giardia lamblia* and the helminth *Strongyloides stercoralis* interfere with the absorption of food across the intestinal mucosa. Hookworm infections cause loss of iron, an essential mineral. Other helminths, such as *Clonorchis sinensis* and *Schistosoma haematobium,* compromise the function of important organs by obstruction, secondary bacterial infection, and induction of carcinomatous changes. Occasionally, as in the case of echinococcosis, disease results from pressure and displacement of normal tissue by the slow growth of the parasitic cyst. In malaria, the primary pathogenic mechanism appears to be the invasion and subsequent alteration or destruction of human erythrocytes. Similarly, many helminthic larvae are capable of tissue invasion and destruction. *Entamoeba histolytica* can destroy host cells without actual cellular invasion. Finally, immunologic mechanisms are responsible for tissue damage and clinical manifestations in many diseases. Allergic or anaphylactic reactions play a major role in the cutaneous reactions to invading hookworm, strongyloides, and schistosome larvae (ground itch, swimmers' itch) and in the fever, rash, and lymphadenopathy that accompany the therapeutic destruction of onchocercal microfilariae (Mazzotti reaction). Transient pneumonias induced by the pulmonary migration of *Ascaris* and other nematode larvae (Loeffler's syndrome), nocturnal paroxysms of asthma in some patients with filariasis (tropical pulmonary eosinophilia), and the shock, asthma, and urticaria that follow rupture of a hydatid cyst are all immunologically mediated. Hemolysis in malaria and cardiac damage in Chagas' disease are thought, at least in part, to reflect antibody-mediated cytotoxicity. Immune complex diseases are seen in schistosomiasis (Katayama syndrome) and malaria (nephrosis). The granulomatous reaction to schistosomal eggs, the muscle damage in trichinosis, and the entire clinicopathologic spectrum of the leishmanial infections appear to be caused by cell-mediated immune responses.

Wide range of direct pathogenic mechanisms

Immunopathologic mechanisms contribute to parasitic diseases

## DIAGNOSIS

Although parasitic diseases are not as common in the United States as elsewhere, they do occur and may, at times, be life threatening. In addition, the continuous arrival of travelers and immigrants from endemic areas necessitates consideration of these diseases in differential diagnoses. Unfortunately, the clinical manifestations of parasitic infections are seldom sufficiently characteristic to raise this possibility in the clinician's mind. Moreover, routine laboratory tests are seldom of aid. Although eosinophilia has been recognized as an important clue to the diagnosis of parasitic disease, this phenomenon is characteristic only of helminthic infection, and even in these cases it is frequently absent. Eosinophilia, which presumably reflects an immunologic response to the complex foreign proteins possessed by worms, is most marked during tissue migration. Once migration ceases, the eosinophilia may decrease or disappear entirely. Thus, the clinician must usually rely on a detailed travel, food intake, transfusion, and socioeconomic history to raise the possibility of parasitic disease.

Need to consider indigenous and imported infections

Eosinophilia seen in helminthic infections

Once considered, diagnosis is usually straightforward. Typically, it rests on the demonstration and morphologic identification of the parasite or its progeny in the stool, urine, sputum, blood, or tissues of the human host.

Morphologic demonstration of parasites primary diagnostic means

In intestinal infections, a simple wet mount or stained smear, or both, of the stool is often adequate. Some parasites, however, are passed in the feces intermittently or in fluctuating numbers, and repeated specimens are needed. Ova of worms and cysts of protozoa may be concentrated by sedimentation or flotation techniques to increase their numbers for diagnosis. Occasionally, specimens other than stool must be examined. In the case of small bowel infections such as giardiasis and strongyloidiasis, aspirates of the duodenum or a small bowel biopsy may be required to establish the diagnosis. Similarly, the recovery of large bowel parasites such as *E. histolytica* and *Schistosoma mansoni* may require proctoscopy or sigmoidoscopy, with aspiration or biopsy of suspect lesions. Eggs of pinworms (*Enterobius*) and tapeworms (*Taenia*) may be found on the perineal skin when they are absent from the stool.

Stool concentration techniques used for intestinal parasites

Parasites dwelling within the tissue and blood of the host are more difficult to identify. Direct examination of the blood is useful for the detection of malarial parasites, leishmania, trypanosomes, and filarial progeny (microfilariae). The concentration of organisms in the bloodstream often fluctuates, however, requiring the collection of multiple specimens over several days. Both wet mount and stained preparations of thin and thick blood smears (see Chapter 52) are used. Lung flukes and occasionally other helminths discharge their offspring in the sputum and may be found there with appropriate concentration techniques. In others, larvae can be recovered with skin (onchocerciasis) or muscle (trichinosis) biopsy.

Demonstration of blood and tissue parasites requires proper timing

In some infections, parasite recovery is uncommon. Immunodiagnostic and nucleic acid hybridization techniques provide diagnostic alternatives for these situations. Although tests for circulating antibodies have long been available for a number of parasitic diseases, they have often lacked sensitivity and specificity. The replacement of crude, antigenically complex parasitic extracts with purified homologous antigens, together with the adaptation of highly reactive test systems, has significantly increased the sensitivity and specificity of such tests. Currently, reliable serologic procedures are available for amebiasis, cysticercosis, echinococciasis, paragonimiasis, schistosomiasis, strongyloidiasis, toxocariasis, toxoplasmosis, and trichinosis. More will undoubtedly follow in the near future.

Serologic tests available for some parasites

Techniques for the detection of parasitic antigens in blood, body fluids, tissues, and excreta also have been developed. Commercial immunofluorescent and immunosorbent kits for *Pneumocystis carinii* (pulmonary secretions), *T. vaginalis* (genitourinary fluids), and *E. histolytica, Giardia,* and *Cryptosporidium* (feces) are now commonly found in clinical laboratories. Less generally available are systems for the detection of malaria antigens in blood and *T. gondii* in tissue.

Antigen detection becoming available

DNA probes are available for the detection of *P. falciparum, T. cruzi, T. brucei, Onchocerca* species, and the etiologic agents of lymphatic filariasis. The probes for *P. falciparum* and lymphatic filariae have demonstrated sensitivities that match or exceed those of traditional techniques. The major limitations of DNA probes as diagnostic tools, relate to the technical aspects of the hybridization procedure which should soon be overcome.

Molecular methods for DNA detection being used with increasing frequency

## CHEMOTHERAPY

The study and management of parasitic disease were seminal to the initiation of the chemotherapeutic era. Amazonian Indians first used quinine-containing extracts of cinchona tree bark to treat malarious patients more than 300 years ago. It was in the attempt to synthesize this same antimalarial compound that 19th-century German chemists discovered aniline dyes. The circle closed in the early years of this century when Ehrlich, while investigating the suitability of these dyes as protozoan stains, developed the concept that chemicals might be found that had the capacity to destroy microbial pathogens selectively without damage to the tissues of the human host. Although the most dramatic confirmation of that concept came with the introduction of arsenical compounds for the treatment of syphilis, his first successful chemotherapeutics were directed against protozoan agents. By 1930, chemically synthesized drugs had been marketed for the treatment of malaria, trypanosomiasis, and schistosomiasis.

Antiparasitic agents among first antimicrobics

The introduction and explosive increase in the number and variety of antimicrobic agents introduced in the latter three fourths of the 20th century forever changed the face of medicine. Unfortunately, however, few were effective against parasites because they share the eukaryotic characteristics of their hosts. With the resources of the pharmaceutical companies directed toward the development and introduction of antibacterial agents, work on antiparasitic agents lagged. Because of the lack of safer alternatives, chemotherapeutics synthesized in the preantibiotic era remained critical elements of the parasitologist's therapeutic armamentarium until very recently. Most required prolonged or parenteral administration, the effectiveness of many was restricted to particular disease stages, and the toxicity of a few mandated that use be limited to very severe or life-threatening conditions. With time, and at a pace much slower than that seen for the antibacterial agents, newer antiparasitic agents were developed that overcame many of these problems. Their numbers are still limited, and only recently has their safety and efficacy begun to match those of their antibacterial equivalents.

Newer antiparasitics have broader spectrum and are less toxic

### Therapeutic Goals

The process of antiparasitic drug development and use has been shaped to a significant degree by the concentration of parasitic diseases in the impoverished areas of the world. Community-based public health measures aimed at interrupting pathogen transmission, such as provision of sanitary facilities and clean water supplies, are still often beyond the capacity of tightly constrained budgets, and the major burden of mitigating the impact of

Treatment programs difficult in underdeveloped economies parasitic illnesses in endemic areas often falls on medical auxiliaries or village health workers who, operating in remote and relatively primitive conditions, must examine, diagnose, and treat sick patients with whom they have only fleeting contact. Given these limitations and the large numbers of the afflicted, optimal therapy requires drugs that are effective in a single dose, easily administered, safe enough to be dispensed with limited medical supervision, and sufficiently inexpensive to be widely used. Few such agents exist. Pharmaceutical companies, faced with the enormous costs of drug development and approval, have been reluctant to expend resources they are unlikely to recover. Until the international community provides the resources needed for the development of more suitable agents, the full potential of antiparasitic chemotherapy will not be realized.

Ideal agents would be inexpensive, of low toxicity, and effective in single doses; few of these exist

The practical aspects of antiparasitic therapy are illustrated in the principles governing the treatment of worm infections, which differ significantly from those applied to prokaryotic or protozoan infections. Helminths, with few exceptions, do not multiply within the human host, and severe infections thus require the repeated acquisition of infectious parasites. Interestingly, the intensity of infection or worm burden does not follow a normal distribution in human populations. Most infected individuals harbor fewer than a dozen adult worms; a small minority harbor very large worm numbers. Because there is a direct correlation between worm burden and clinical disease, only this minority suffers significant morbidity. Concentrating treatment on those few clinically ill patients moderates the medical impact of a helminthic disease on a community at a cost dramatically lower than that required for mass treatment. Moreover, it is usually unnecessary to eradicate all worms from treated patients; a significant decrease in the worm burden is adequate to alleviate clinical symptoms. This can often be accomplished with short, subcurative doses that further reduce cost and minimize the likelihood of drug toxicity. Because this approach can dramatically decrease the total community worm burden, the number of worm progeny shed into the environment is similarly reduced and the transmission of the disease slowed or, at times, eliminated.

For worms, treatment efforts should be concentrated on the most heavily parasitized individuals

## Structure and Action

With few exceptions, antiparasitic agents have been synthesized de novo rather than developed from naturally occurring substances. Most are relatively simple and often contain benzene or other ring structures.

Most antiparasitics are synthetic

It is believed that the majority of antiprotozoan drugs interfere with nucleic acid synthesis or, less commonly, with carbohydrate metabolism. Anthelmintics, on the other hand, apparently act by compromising the worm's glycolytic pathways or neuromuscular function. In most cases, the parasite and host cells have functionally equivalent target sites. Differential toxicity is achieved by preferential uptake, metabolic alteration of the drug by the parasite, or differences in the susceptibility of functionally equivalent sites in parasite and host.

Differential toxicity based on uptake, metabolic factors

As has been the case for antibacterial agents, the impact of many antiparasitic agents has been compromised by the development of resistance in the parasite. This seems to have resulted from mutation and selection in the face of intensive, often prophylactic, drug use. The mechanisms responsible have been studied for only a few parasites, but appear to be related to reduced uptake of drug.

Acquired mutational resistance usually involves reduced uptake of drug

## Drugs

### *Heavy Metals*

Arsenic and antimonial compounds have been used since ancient times. They form stable complexes with sulfur compounds and probably exert their biological effects by binding to sulfhydryl groups. They are toxic to the host as well as to the parasite and have their greatest impact on cells that are most metabolically active such as neuronal, renal tubular, intestinal epithelial, and bone marrow stem cells. Their differential toxicity and therapeutic value are due to enhanced uptake by the parasite and its intense metabolic activity. Only one trivalent arsenical, melarsoprol* (Mel B), is now widely used. It is capable of

Arsenic and antimonial compounds inactivate —SH groups

Differential toxicity based on enhanced uptake by parasite

* Available from the Centers for Disease Control and Prevention Drug Service.

Melarsoprol is active against all stages of trypanosomiasis

penetrating the blood–brain barrier and is effective in all stages of trypanosomiasis. Because of its toxicity, it is employed only when less toxic agents have failed or the central nervous system is involved. The recently introduced less toxic trypanocides that penetrate the blood–brain barrier may soon replace this drug.

Antimonials used only for leishmanial infections

Antimonial agents are now restricted to the management of leishmanial infections. Two pentavalent compounds, sodium stibogluconate* (Pentostam) and meglumine antimoniate† (Glucantime), are used for all forms of leishmaniasis. In disseminated disease, prolonged therapy is usually required and relapses often occur. In localized cutaneous leishmaniasis, cure is usually achieved with a relatively brief course. Toxic side effects are similar to those of the arsenicals.

## *Antimalarial Quinolines*

Quinine and quinoline analogs active against malaria

Cinchona bark was used in Europe for the treatment of fever as early as 1640. Only after Pelletier and Caventou isolated quinine from cinchona in 1820 did this alkaloid gain widespread acceptance as an antimalarial. Synthesis of new quinolines was stimulated by the interruption of quinine supplies during World Wars I and II and, after 1961, by the growing impact of drug-resistant falciparum malaria in several areas of the world. Among the most effective agents are those that share the double-ring structure of quinine.

Accumulate in parasitized cells and block DNA synthesis

Current analogs fall into three major groups: 4-aminoquinolines, 8-aminoquinolines, and 4-quinolinemethanols. Selective destruction of intracellular parasites results from accumulation of the quinolines by parasitized host cells. Most of these agents appear to block nucleic acid synthesis by intercalation into double-stranded DNA. However, the failure of the 4-quinolinemethanols to intercalate indicates that other mechanisms, perhaps inhibition of heme polymerase, with the build up of toxic hemoglobin metabolites within the malarial parasite, are involved.

Quinine, 4-aminoquinolines (eg, chloroquine), and 4-quinolinemethanols suppress malarial infection

8-Aminoquinolines (eg, primaquine) effect radical cure

Quinine, 4-aminoquinolines, and 4-quinolinemethanols are preferentially concentrated in parasitized erythrocytes and rapidly destroy the erythrocytic stage of the parasite that is responsible for the clinical manifestations of malaria. Thus, these agents can be used either prophylactically to suppress clinical illness should infection occur or therapeutically to terminate an acute attack. They do not concentrate in tissue cells, and thus organisms sequestered in exoerythrocytic sites, particularly the liver, survive and may later reestablish erythrocytic infection and produce a clinical relapse. The 8-aminoquinolines accumulate in tissue cells, destroy hepatic parasites, and effect a radical cure.

Primaquine has hematologic toxicity

Chloroquine phosphate, a 4-aminoquinoline, is the most widely used of the blood schizonticidal drugs. In the doses used for long-term malarial prophylaxis, it has proven remarkably free of untoward effects. Primaquine phosphate, the 8-aminoquinoline used to eradicate persistent hepatic parasites, has toxic effects related to its oxidant activity. Methemoglobinemia and hemolytic anemia are particularly frequent in patients with glucose-6-phosphate dehydrogenase deficiency, because they are unable to generate sufficient quantities of the reduced form of nicotinamide adenine dinucleotide to respond to this oxidant stress. Typically, the anemia is severe in patients of Mediterranean and Far Eastern ancestry and mild in black patients.

Quinine is active against many chloroquine-resistant malarial strains

Quinine is the most toxic of the quinolines and is currently used primarily to treat the strains of *P. falciparum* resistant to several blood schizonticidal agents that are spreading rapidly through Asia, Latin America, and Africa. Chloroquine resistance is the most frequent and worrisome, because suitable alternatives to this safe and highly effective agent are few. The mechanism of resistance is not clearly understood, but resistant organisms fail to accumulate chloroquine. Experimental reversal of resistance with calcium channel blockers suggests that the failure to accumulate this agent results from a rapid release mechanism. Quinidine, a less cardiotoxic optical isomer of quinine, is more readily available in the United States and is preferred to quinine when parenteral administration is required. Mefloquine, a more recently developed oral 4-quinolinemethanol, originally displayed a high level of activity against most chloroquine-resistant parasites; however, mefloquine-resistant

* Available from the Centers for Disease Control and Prevention Drug Service.

† Not available in the United States.

strains of *P. falciparum* are now widespread in Southeast Asia, and present, to a lesser degree, in South America. Resistant strains have recently been identified in Africa.

Phenanthrene methanols are not, in the strict sense, quinine analogs. Nevertheless, they are structurally similar to this group of agents and, together with them, were discovered to have antimalarial activity during the second World War. Halofantrine†, the most effective of the group, has only recently become available. In vitro and in vivo studies demonstrated that it is an effective blood schizonticide against both sensitive and multidrug-resistant strains of *P. falciparum*. Its mechanism of action was originally thought to differ from that of quinine and mefloquine. Recently, mefloquine-resistant strains of *P. falciparum* have demonstrated decreased sensitivity to halofantrine, raising the possibility of cross-resistance between these two agents. Rarely, halofantrine has produced fatal heart arrhythmias, and it should not be given to patients with cardiac conduction abnormalities. It is otherwise well tolerated and appears to be free of teratogenicity. Oral absorption is both slow and erratic, reaching maximum concentrations in 5 to 7 hours; its half-life is relatively short (1 to 3 days). Clinical studies have demonstrated high failure rates when the drug is given in a single dose; cure rates with multiple-dose regimens, however, have been high.

Phenanthrene methanols active against multidrug-resistant malaria

## *Quinones*

Atovaquone is a novel hydroxynaphthoquinone that shows promise in the treatment of malaria and toxoplasmosis. In the search for effective antimalarial agents during World War II, a number of hydroxynaphthoquinones were found to have antimalarial activity in experimental animals; however, all were rapidly metabolized in humans and proved ineffective in the treatment of malarious patients. In the 1980s a single hydroxynaphthoquinone, atovaquone, was found to be both highly effective in vitro against *P. falciparum* and metabolically stable in humans when administered orally. Its antiparasitic activity appears to result from the specific blockade of pyrimidine biosynthesis secondary to the inhibition of the parasite's mitochondrial electron transport chain at the ubiquinol–cytochrome c reductase region (complex III). Its long half-life (70 hours) and lack of serious adverse reactions suggested that it would be of great value in the treatment of malaria. Efficacy trials established its capacity to effect rapid clearance of parasitemia in patients with chloroquine-resistant falciparum malaria. Frequent parasitic recrudescences were eliminated when atovaquone was administered in combination with proguanil or tetracycline. Subsequently, this agent has shown to be effective for the treatment of toxoplasmosis in patients with acquired immunodeficiency syndrome (AIDS). Unlike other antitoxoplasma agents, atovaquone has been found to be active against *T. gondii* cysts as well as tachyzoites, suggesting this agent may produce radical cure. Supporting this is the infrequency with which cessation of atovaquone treatment of toxoplasmic cerebritis in AIDS patients has resulted in relapse. Relapse following atovaquone treatment of pneumocystosis in this same patient population appears similarly uncommon.

Atovaquone stable and active against malaria and toxoplasmosis

## *Folate Antagonists*

Folic acid serves as a critical coenzyme for the synthesis of purines and ultimately DNA. In protozoa, as in bacteria, the active form of folic acid is produced in vivo by a simple two-step process. The first, the conversion of *para*-aminobenzoic acid to dihydrofolic acid, is blocked by sulfonamides. The second, the transformation of dihydro- to tetrahydrofolic acid, is inhibited by folic acid analogs (folate antagonists), which competitively inhibit dihydrofolate reductase. Used together with sulfonamides, folate antagonists are very effective inhibitors of protozoan growth.

Sulfonamide and folate antagonists inhibit protozoa

Trimethoprim, an inhibitor of dihydrofolate reductase, is used in combination with sulfamethoxazole to treat toxoplasmosis. Another folate antagonist, pyrimethamine, has a high affinity for sporozoan dihydrofolate reductase and has been particularly effective, when used with a sulfonamide, in the management of clinical malaria and toxoplasmosis.

Trimethoprim effective in *Toxoplasma* and *Pneumocystis* infections

† Not available in the United States.

In East Africa, a third folate antagonist, proguanil, is commonly taken in combination with chloroquine for malaria prophylaxis. Acquired protozoal resistance to folate antagonists is mutational and generally has been limited to particular species of malarial parasites.

Folate deficiency and sulfonamide toxicities occur

Folate antagonists may result in folate deficiency in individuals with limited folate reserves, such as newborns, pregnant women, and the malnourished. This is of great concern when large doses are used for prolonged periods, as in the treatment of acute toxoplasmosis. When folate antagonists are used with sulfonamides, the entire range of sulfonamide toxic effects may be seen. Patients with AIDS appear to suffer an unusually high incidence of toxic side effects to trimethoprim–sulfamethoxazole.

## *Qinghaosu (Artemisinin†)*

Plant derivative active against malaria, amebas, and *Schistosoma*

Concentrated in parasitized erythrocytes

This natural extract of the plant *Artemisia annua* (qing hao, sweet wormwood) is a sesquiterpenelactone peroxide that is structurally distinct from all other known antiparasitic compounds. Extracts of qing hao were recommended for the treatment of fevers in China as early as AD 341; their specific antimalarial activity was defined in 1971. Although qinghaosu has also been shown to be active against the free-living ameba *Naegleria fowleria* and several trematodes, including *Schistosoma japonicum, Schistosoma mansoni,* and *Clonorchis sinensis,* its greatest impact to date has been in the treatment of malaria. Extensive investigations showed it to be schizonticidal for both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. Several derivatives, among them artemether† and artesunate, are significantly more active than the parent compound. All are concentrated in parasitized erythrocytes where they decompose, releasing free radicals, which are thought to be damaging to parasitic membranes. Artemisinin compounds act more rapidly than other antimalarial agents, stopping parasite development and preventing cytoadherence in falciparum malaria. Although depression of reticulocyte counts has been noted, these agents appear significantly less toxic than quinoline antimalarials. As there is some evidence that they may possess teratogenic properties, they should not be used in pregnancy. Importantly, they may be given orally, rectally (by suppository), and parenterally. Relapses can occur unless they are given for several days or combined with a second agent such as mefloquine or tetracycline.

## *Nitroimidazoles*

Active against protozoa at low redox potential

Metronidazole, a nitroimidazole, was introduced in 1959 for the treatment of trichomoniasis. Subsequently, it was found to be effective in the management of giardiasis, amebiasis, and a variety of infections produced by obligate anaerobic bacteria. Energy metabolism in all of them depends on the presence of low-redox-potential compounds, such as ferredoxin, to serve as electron carriers. These compounds reduce the 5-nitro group of the imidazoles to produce intermediate products responsible for the death of the protozoal and bacterial cells, possibly by alkylation of DNA. Resistance, although uncommon, has been noted in strains of *T. vaginalis* lacking nitroreductase activity. Of greater concern is in vitro evidence of mutagenicity. Metronidazole is the drug of choice for trichomoniasis and invasive amebiasis. It is effective in giardiasis although not yet approved by the Food and Drug Administration for use in this infection. Tinidazole, a newer nitroimidazole not yet available in the United States, appears to be both a more effective and less mutagenic antiprotozoal agent. Its greater lipid solubility improves cerebrospinal fluid levels and in vitro activity.

## *Benzimidazoles*

Broad-spectrum anthelmintics

As the name **benzimidazole** implies, the basic structure of these antiparasitic agents consists of linked imidazole and benzene rings. Unlike their antiprotozoal cousins discussed above, the benzimidazoles are broad-spectrum anthelmintic agents. The prototype drug,

† Not available in the United States.

thiabendazole, acts against both adult and larval nematodes and was shown to be useful in the management of cutaneous larva migrans, trichinosis, and most intestinal nematode infections soon after its introduction in the early 1960s. The mechanism by which it exerts its anthelmintic action is uncertain. It is known to inhibit fumarate reductase, an important mitochondrial enzyme of helminths. The primary mode of action, however, may derive from the known capacity of all benzimidazoles to inhibit the polymerization of tubulin, the eukaryotic cytoskeletal protein, as described for mebendazole below. Side effects are mild, related to the gastrointestinal tract or liver, and rapidly disappear with the discontinuation of the drug. Hypersensitivity reactions, induced either by the drug or by antigens released from the damaged parasite, may occur.

Inhibit helminth fumarate reductase

Mebendazole, a carbamate benzimidazole introduced in 1972, has a spectrum similar to that of thiabendazole, but also has been found to be effective against a number of cestodes, including *Taenia, Hymenolepsis,* and *Echinococcus*. It irreversibly blocks glucose uptake of both adult and larval worms, resulting in glycogen depletion, cessation of ATP formation, and paralysis or death. It does not appear to affect glucose metabolism in humans and is thought to exert its effect in worms by binding to tubulin, thus interfering with the assembly of cytoplasmic microtubules, structures essential to glucose uptake. Unlike thiabendazole, the drug is not well absorbed from the gastrointestinal tract and may owe part of its effectiveness against intestine-dwelling adult worms to its high concentrations in the gut. Toxicity is uncommon. Teratogenic effects have been observed in experimental animals; its use in infants and pregnant women is contraindicated.

Mebendazole blocks glucose uptake by adult and larval worms

Interferes with tubulin and cytoplasmic microtubules

Albendazole is a benzimidazole carbamate that has recently been made available in the United States. It has a somewhat broader spectrum than that of its close relative, mebendazole, being more active against *Strongyloides stercoralis* and several tissue nematodes. In addition to the vermicidal and larvicidal properties that it shares with other benzimidazoles, it is ovicidal, enhancing its effectiveness in tissue cestode infections such as echinococciasis and cysticercosis. Its activity against *Giardia,* one of the most common intestinal protozoa, makes it an appealing candidate for the treatment of polyparasitism. Although it shares the teratogenic potential of other benzimidazoles, it is otherwise extremely well tolerated. Single-dose therapy is effective in the management of many intestinal nematode infections.

Albendazole has broader spectrum

## *Avermectins*

Avermectins are macrocyclic lactones produced as fermentation products of *Streptomyces avermitilis*. Structurally similar to the macrolide antibiotics, they are effective at extremely low concentration against a wide variety of nematodes and arthropods. The avermectins appear to induce neuromuscular paralysis by acting on a receptor of the parasites-peripheral neurotransmitter, gamma-aminobutyric acid (GABA). In mammals, GABA is confined to the central nervous system, and because the avermectins do not cross the blood–brain barrier in significant concentration, they do not appear to produce significant untoward effects in the mammalian host. Ivermectin, a derivative of avermectin B1, is currently the drug of choice for the treatment of onchocerciasis and is undergoing evaluation for the treatment of other human filarial infections. Its usefulness in other parasitic infections of humans remains to be established.

Antibiotics that influence nematode neurotransmitters

Activity against filariae

## *Praziquantel*

Praziquantel, a heterocyclic pyrazinoisoquinoline, is an important new anthelmintic effective against a broad range of cestodes and trematodes, many of which had been poorly responsive to previously available agents. It is given in one to three doses. The drug is rapidly taken up by susceptible helminths, in which it appears to induce the loss of intracellular calcium, tetanic muscular contraction, and destruction of the tegument. The differential toxicity of this agent may be related to the inability of susceptible worms to metabolize the drug. Aside from transient, mild gastrointestinal symptoms, praziquantel appears remarkably free of side effects in humans. It is currently the drug of choice for the treatment of schistosomiasis, clonorchiasis, opisthorchiasis, and neurocysticercosis.

Causes loss of intracellular calcium in cestodes and trematodes

Safety of praziquantel allows use in mass therapy campaigns

TABLE 51–5

**Miscellaneous Antiparasitic Agents**

| Compound | Drug Class | Route | Mechanism of Action | Clinical Use | Comments |
|---|---|---|---|---|---|
| Bithionol | Phenol | Oral | Uncouples phosphorylation | Paragonimiasis | Not commercially available in United States |
| Diethylcarbamazine | Piperazine | Oral | Neuromuscular paralysis | Filarial infections | Allergic reactions to filarial antigens |
| Diloxanide furoate | Acetanilide | Oral | Unknown | Intestinal amebiasis | Used only for asymptomatic carriers |
| Iodoquinol (diiodohydroxyquin) | Halogenated quinoline | Oral | Unknown | Intestinal amebiasis<br>*Dientamoeba* infections | Related drug has caused optic atrophy |
| Nifurtimox | Nitrofuran | Oral | Alkylates DNA | Acute Chagas' disease | Toxicity<br>Prolonged therapy<br>Marginal effectiveness |
| Paromomycin | Aminoglycoside | Oral | Similar to other aminoglycosides | Intestinal cryptosporidiosis | Not absorbed<br>Marginal effectiveness |
| Pentamidine | Diamidine | IV | Binds DNA | Leishmaniasis<br>Trypanosomiasis | Toxic |
| Pyrantel pamoate | Tetrahydropyrimidine | Oral | Neuromuscular blockade; inhibits fumarate reductase | Pinworm infection, hookworm infection<br>Ascariasis | Single-dose therapy |
| Spiramycin | Macrolide | Oral | Blocks protein synthesis | Toxoplasmosis | Used to treat pregnant women |
| Suramin | Sulfated naphthylamine | IV | Inhibits glycerophosphate oxidase and dehydrogenase | African trypanosomiasis<br>Onchocerciasis | Not effective in central nervous system disease<br>Renal toxicity |

*Abbreviation:* IV, intravenous.

Good activity has been demonstrated against other common trematode and cestode infections. Its high level of safety suggests that it may well play a significant role in worldwide mass therapy campaigns.

### *Eflornithine*[†] *(Difluoromethylornithine)*

Eflornithine is a specific, enzyme-activated, irreversible inhibitor of ornithine decarboxylase (ODC). In mammalian cells, decarboxylation of ornithine by ODC is a mandatory step in the synthesis of polyamines, compounds thought to play critical roles in cell division and differentiation. Originally developed as an antineoplastic agent, eflornithine proved ineffective in cancer chemotherapy trials. With the discovery that polyamines of *Trypanosoma* species were also synthesized from ornithine, eflornithine was successfully tested in the treatment of animal trypanosomiasis. Host survival was high and associated with decreases in parasitic polyamines and inhibition of nucleic acid synthesis. In the dosage required to treat trypanosomiasis, mammals tolerated the agent well, presumably because *T. brucei* is 100 times more sensitive to the effects of eflornithine than are mammalian cells. Eflornithine appears to be cytostatic and requires an intact host immune system for maximum effect.

Originally an anticancer drug

Active against trypanosomes

### *Other Antiparasitic Agents*

A number of antiparasitic agents used in therapy, their properties, and their clinical uses are listed in Table 51–5.

## CONTROL

The control of diseases spread by the fecal–oral route depends on the improvements in personal hygiene and sanitation that accompany general economic development. In contrast, efforts at preventing the spread of multihost parasites is usually focused on the simultaneous treatment of infected humans and control or elimination of the nonhuman host.

To be effective, such measures must be applied in a comprehensive and coordinated manner over large areas. Administrative problems, political imbroglios, development of resistance in parasites and intermediate hosts, technical difficulties, and funding shortages have, individually and together, limited the success of such efforts. A case in point was the failure of the worldwide malaria eradication effort launched by the World Health Organization in 1955. This has refocused attention on alternative control measures, including immunization. Until recently, the development of effective parasitic vaccines has been constrained by the complexities of their immunologic interactions with the human host. Monoclonal antibodies have helped identify antigens responsible for the induction of immunity to a number of parasitic infections, including malaria, leishmaniasis, and schistosomiasis. The subsequent cloning of the structural genes encoding such antigens has made a large-scale production of vaccine antigen feasible. It is further possible that the entire step of antigen production and purification could be bypassed by the use of synthetic peptide or anti-idiotype vaccines. All these approaches are currently being developed. Malaria vaccines are undergoing clinical trials.

Epidemiologic control in developing countries complex

Vaccines using antigens or synthesized peptides in development

## ADDITIONAL READING

Armitage KB. Antiparasitic drugs and therapy. In: *Clinical Infectious Diseases,* Root RK, Waldvogel F, Corey L, et al. New York: Oxford University Press; 1999, pp 365–388. A recent, concise discussion of this subject.

Desowitz RS. *New Guinea Tapeworms and Jewish Grandmothers: Tales of Parasites and Peoples*. New York: Norton; 1981. A delightful look at the host–parasite relationship.

[†] Not available in the United States.

Drugs for parasitic infections. *Med Lett Drugs Ther* 2002;44:1–12. Concise guide to treatment of parasitic diseases, updated annually.

Horton RJ. Benzimidazole anthelmintics. *Parasitol Today* 1990;6:105–136. The best recent review of this important group of chemotherapeutic agents.

Liu LX, Weller PF. Antiparasitic drugs. *N Engl J Med* 1995;334:1178–1184. A concise review.

Maddison SE. Serodiagnosis of parasitic diseases. *Clin Microbiol Rev* 1991;4:457–469. A short but comprehensive review of current antibody and antigen detection procedures, use of monoclonal antibodies in serodiagnosis, molecular biological technology, and skin tests for parasitic diseases.

Markell EK, John DT, Krotoski WA. Parasites, parasitism, and host relations. In *Markell and Voge's Medical Parasitology,* 8th ed. Philadelphia: WB Saunders; 1999.

Stoll NR. This wormy world. *J Parasitol* 1947;33:1–18. This classic of quantitative helminth epidemiology has recently been updated by DAP Bundy (*Parasitol Today* 1997;13:407–408).

Warren KS (ed). *Immunology and Molecular Biology of Parasitic Infections*. 3rd ed. Boston: Blackwell Scientific; 1993. This relatively comprehensive monograph discusses general immune responses to parasitic infections as well as the immunity, immunopathology, immunodiagnosis, and molecular biology of specific parasitic diseases.

CHAPTER 52

# Sporozoa

JAMES J. PLORDE

Sporozoa are a unique class of intracellular protozoa distinguished by their alternating cycles of sexual and asexual reproduction. Asexual multiplication occurs by a process of multiple fission termed schizogony. The nucleus of a trophozoite divides into several parts, forming a multinucleated schizont. Cytoplasm then condenses around each nuclear portion to form new daughter cells, or merozoites, which burst from their intracellular location to invade new host cells. After the completion of one or more of these asexual cycles, some merozoites differentiate into male and female gametocytes, initiating the cycle of sexual reproduction known as sporogony. The gametocytes mature and effect fertilization, forming a zygote. On encysting, the zygote is known as an oocyst. Sporozoites formed within the oocyst are released, penetrate host tissue cells, and begin another asexual cycle as trophozoites.

Intracellular protozoa with alternating sexual and asexual cycles

Two sporozoan infections, malaria and toxoplasmosis, are common diseases of humans; together, they affect more than one third of the world's population and kill or deform perhaps a million neonates and children each year. A third infection, cryptosporidiosis, has only recently been found to be an important cause of diarrhea, particularly in immunocompromised hosts.

Cause malaria, toxoplasmosis, and cryptosporidiosis

## PLASMODIA

> Of all infectious diseases there is no doubt that malaria has caused the greatest harm to the greatest number.
>
> LADERMAN, 1975

## PARASITOLOGY

### DEFINITION

The plasmodia are sporozoa in which the sexual and asexual cycles of reproduction are completed in different host species. The sexual phase occurs within the gut of mosquitoes. These arthropods subsequently transmit the parasite while feeding on a vertebrate host. Within the red blood cells (RBCs) of the vertebrate, the plasmodia reproduce asexually; they eventually burst from the erythrocyte and invade other uninvolved RBCs. This

Sexual phase in mosquito and asexual phase in humans

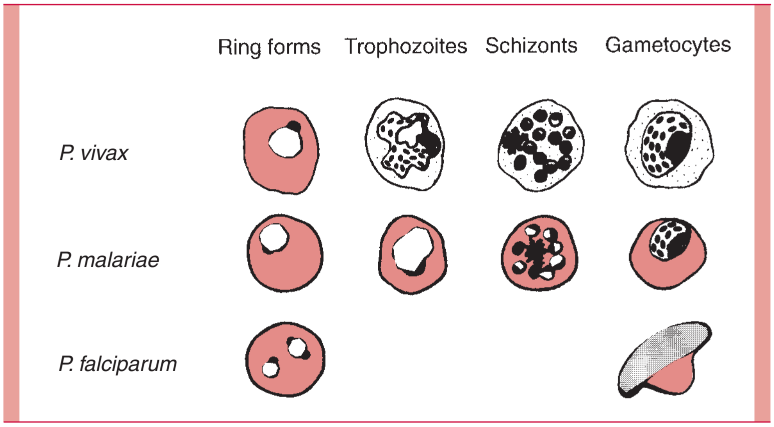



FIGURE 52–1
Examples of erythrocytic stages of malarial parasites. Note: Trophozoite and schizont forms of *Plasmodium falciparum* occur in visceral capillaries rather than in blood. Male and female gametophytes show distinctive morphologic differences.

Four species infect humans

event produces periodic fever and anemia in the host, a disease process known as malaria. Of the many species of plasmodia, four are known to infect humans and will be considered here: *Plasmodium vivax, P. ovale, P. malariae,* and *P. falciparum*.

## MORPHOLOGY

Morphology of the parasite and the infected RBC vary by stage and species

The morphology of the stained intraerythrocytic parasites is shown in Figure 52–1. In stained smears, three characteristic features aid in the identification of plasmodia: red nuclear chromatin; blue cytoplasm; and brownish-black malarial pigment, or hemozoin, consisting largely of a hemoglobin degradation product, ferriprotoporphyrin IX. The change in the shape of the cytoplasm and the division of the chromatin at different stages of parasite development are obvious. Gametocytes can be differentiated from the asexual forms by their large size and lack of nuclear division. Some of the infected erythrocytes develop membrane invaginations or caveolae–vesicle complexes, which are thought to be responsible for the appearance of the pink Schüffner's dots or granules (see below).

Morphologic differences are the primary means of diagnosis

The appearance of each of the four species of plasmodia that infect humans is sufficiently different to allow their differentiation in stained smears. The parasitized erythrocyte in *P. vivax* and *P. ovale* infections is pale, enlarged, and contains numerous Schüffner's dots. All asexual stages (trophozoite, schizont, merozoite) may be seen simultaneously. Cells infected by *P. ovale* are elongated and frequently irregular or fimbriated in appearance. In *P. malariae* infections, the RBCs are not enlarged and contain no granules. The trophozoites often present as "band" forms, and the merozoites are arranged in rosettes around a clump of central pigment. In *P. falciparum* infections, the rings are very small and may contain two chromatin dots rather than one. There is often more than one parasite per cell, and parasites are frequently seen lying against the margin of the cell. Intracytoplasmic granules known as Maurer's dots may be present but are often cleft shaped and fewer in number than Schüffner's dots. Schizonts and merozoites are not present in the peripheral blood. Gametocytes are large and banana shaped. These characteristics are summarized in Table 52–1.

## LIFE CYCLE OF MALARIAL PARASITES

Mosquito ingests gametocytes from blood of infected human

Sporozoites from oocyst reach mosquito salivary glands

Sporogony, or the sexual cycle, begins when a female mosquito of the genus *Anopheles* ingests circulating male and female gametocytes while feeding on a malarious human. In the gut of the mosquito, the gametocytes mature and effect fertilization. The resulting zygote penetrates the mosquito's gut wall, lodges beneath the basement membrane, and vacuolates to form an oocyst. Within this structure, thousands of sporozoites are formed. The enlarging cyst eventually ruptures, releasing the sporozoites into the body cavity of the mosquito. Some penetrate the salivary glands, rendering the mosquito infectious for humans. The time required for the completion of the cycle in mosquitoes varies from 1 to

TABLE 52–1

**Differential Characteristics of *Plasmodium* Species**

| CHARACTERISTICS | *P. VIVAX* | *P. OVALE* | *P. MALARIAE* | *P. FALCIPARUM* |
|---|---|---|---|---|
| Erythrocyte | | | | |
| Enlarged, pale | + | + | − | − |
| Oval, fimbriated | − | + | − | − |
| Schüffner's dots | + | + | − | − |
| Maurer's dots | − | − | − | + |
| Parasite | | | | |
| All asexual stages seen | + | + | + | − |
| Band forms | − | − | + | − |
| Double infections | − | − | − | + |
| Double chromatin dots | − | − | − | + |
| Banana-shaped gametocytes | − | − | − | + |

3 weeks, depending on the species of insect and parasite as well as on the ambient temperature and humidity.

Schizogony, the asexual cycle, occurs in the human and begins when the infected *Anopheles* takes a blood meal from another individual. Sporozoites from the mosquito's salivary glands are injected into the human's subcutaneous capillaries and circulate in the peripheral blood. Within 1 hour they attach to and invade liver cells (hepatocytes), a process thought to be mediated by a ligand present in the sporozoites' outer protein coat (circumsporozoite protein). In *P. vivax* and *P. ovale* infections, some of the sporozoites enter a dormant state immediately after cell invasion. The remaining sporozoites initiate exoerythrocytic schizogony, each producing about 2000 to 40,000 daughter cells, or merozoites. One to two weeks later, the infected hepatocytes rupture, releasing merozoites into the general circulation.

Humans infected by mosquito bite

Rapid infection of hepatocytes starts asexual cycle in humans

The erythrocytic phase of malaria starts with the attachment of a released hepatic merozoite to a specific receptor on the RBC surface. After attachment, the merozoite invaginates the cell membrane and is slowly endocytosed. The intracellular parasite initially appears as a ring-shaped trophozoite, which enlarges and becomes more active and irregular in outline. Within a few hours, nuclear division occurs, producing the multinucleated schizont. Cytoplasm eventually condenses around each nucleus of the schizont to form an intraerythrocytic cluster of 6 to 24 merozoite daughter cells. About 48 (*P. vivax, P. ovale,* and *P. falciparum*) to 72 (*P. malariae*) hours after initial invasion, infected erythrocytes rupture, releasing the merozoites and producing the first clinical manifestations of disease. The newly released daughter cells invade other RBCs, where most repeat the asexual cycle. Other daughter cells are transformed into sexual forms or gametocytes. These latter forms do not produce RBC lysis, and continue to circulate in the peripheral vasculature until ingested by an appropriate mosquito. The recurring asexual cycles continue, involving an ever-increasing number of erythrocytes until finally the development of host immunity brings the erythrocytic cycle to a close. The dormant hepatic sporozoites of *P. vivax* and *P. ovale* survive the host's immunologic attack, and may, after a latent period of months to years, resume intrahepatic multiplication. This leads to a second release of hepatic merozoites and the initiation of another erythrocytic cycle, a phenomenon known as relapse. The life cycle of malarial parasites is summarized in Figure 52–2.

Erythrocytic cycle begins with merozoite attachment to RBC receptor

Trophozoites multiply in RBC to form new merozoites

In 48–72 hours, RBCs rupture, releasing merozoites to infect new RBCs

Intrahepatic dormancy causes relapses with *P. vivax* and *P. ovale*

## PHYSIOLOGY

Species of plasmodia differ significantly in their ability to invade subpopulations of erythrocytes; *P. vivax* and *P. ovale* attack only immature cells (reticulocytes), whereas *P.*

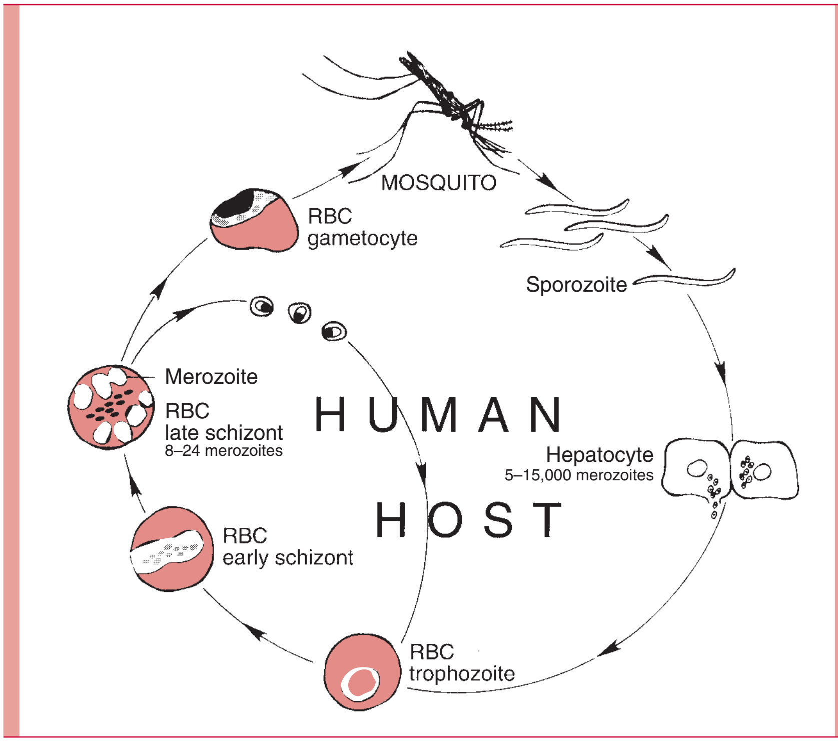



FIGURE 52–2
Life cycle of the malarial parasite. RBC = red blood cell.

*malariae* attacks only senescent cells. During infection with these species, therefore, no more than 1 to 2% of the cell population is involved. *P. falciparum,* in contrast, invades RBCs regardless of age and may produce very high levels of parasitemia and particularly serious disease. In part, these differences may be related to the known differences in the RBC receptor sites available to the individual *Plasmodium* species. In the case of *P. vivax,* the site is closely related to the Duffy blood group antigens ($Fy^a$ and $Fy^b$). Duffy-negative individuals, who constitute the majority of people of West African ancestry, are therefore resistant to vivax malaria. RBC sialoglycoprotein, particularly glycoprotein A, has been implicated as the *P. falciparum* receptor site.

Parasites vary in ability to attack subpopulations of erythrocytes

RBC Duffy antigen and glycoprotein A are RBC receptors

Certain RBC abnormalities may also effect parasitism. The altered hemoglobin (hemoglobin S) associated with the sickle cell trait limits the intensity of the parasitemia caused by *P. falciparum,* and thereby provides a selective advantage to individuals who are heterozygous for the sickle cell gene. As a result, the sickle cell gene, which would otherwise be disadvantageous, is found at high frequency in populations living in malarious areas. Parasite growth appears to be retarded in RBCs heterozygous for hemoglobin S (SA) when they are exposed to conditions of reduced oxygen tension such as might be present in the visceral capillaries. Sickling may also render the erythrocyte more susceptible to phagocytosis or directly damage the parasite. A similar protective effect may be exerted by hemoglobins C, D, and E; thalassemias; and glucose-6-phosphate dehydrogenase (G6PD) or pyridoxal kinase deficiencies, because these abnormalities have also been found more frequently in malarious areas. The protection in these conditions may be related to the increased susceptibility of such RBCs to oxidant stress. In thalassemia, the protection may also be related in part to the production of fetal hemoglobin, which retards maturation of *P. falciparum,* as well as an increased binding of antibodies to modified parasitic antigens (neoantigens) presenting on the surface of the erythrocytes.

Sickle cell trait limits intensity of *P. falciparum* infection

Other hemoglobinopathies can also exert protection

Once invasion has occurred, malaria parasites may induce a number of changes in the erythrocytic membrane. These include alteration of its lipid concentration, modification of its osmotic properties, and incorporation of parasitic neoantigens, rendering the RBC susceptible to immunologic attack. *P. vivax* and *P. ovale* stimulate the production of caveolae–vesicle complexes, which are visualized as Schüffner's dots in stained smears. In *P. falciparum* infections, electron-dense elevated knobs or excrescences form on the RBC

Changes induced in erythrocyte membrane

Binding to endothelium may cause microinfarcts surface. These produce a strain specific, high-molecular-weight adhesive protein (PfEMP1), which mediates binding to receptors on the endothelium of capillaries and postcapillary venules of the brain, placenta, and other organs, where they can produce obstruction and microinfarcts.

Malarial parasites metabolize anaerobically, synthesize their own folate

Malarial parasites generate energy by the anaerobic metabolism of glucose. They appear to satisfy their protein requirements by the degradation of hemoglobin within their acidic food vacuoles, resulting in the formation of the malarial pigment (hemozoin) mentioned previously. It has been estimated that the average plasmodium destroys between 25 and 75% of the hemoglobin of its host erythrocyte. Unlike their vertebrate hosts, malarial parasites synthesize folates de novo. As a result, antifolate antimicrobics such as pyrimethamine are effective antimalarial agents.

## GROWTH IN THE LABORATORY

Continuous in vitro cultivation of plasmodia in human erythrocytes was first achieved in 1976. More recently, the successful in vitro completion of the entire sporogonic cycle, from ookinete to sporozoite, has been achieved. These twin developments provide new opportunities for studying the biology, immunology, and chemotherapy of human malaria. The most immediate impact of these advances has been on the introduction of methods for testing the sensitivity of *P. falciparum* to chemotherapeutic agents. Ultimately, these agents will play critical roles in the development of effective antimalarial vaccines.

# MALARIA

CLINICAL CAPSULE

Malaria is a febrile illness caused by a parasitic infection of human erythrocytes transmitted by the bite of a mosquito. The fevers are accompanied by headache, sweats, malaise, and typically appear in paroxysmal episodes lasting hours and recurring for weeks. Complications due to capillary blockade can be fatal, particularly in the brain.

## EPIDEMIOLOGY

Distribution in tropical areas worldwide

Malaria has a worldwide distribution between 45°N and 40°S latitude, generally at altitudes below 1800 m. *P. vivax* is the most widely distributed of the four species, and together with the uncommon *P. malariae,* is found primarily in temperate and subtropical areas. *P. falciparum* is the dominant organism of the tropics. *P. ovale* is rare and found principally in Africa.

Clinical manifestations muted with hyperendemicity

The intensity of malarial transmission in an endemic area depends on the density and feeding habits of suitable mosquito vectors and the prevalence of infected humans, who serve as parasite reservoirs. In hyperendemic areas (areas where more than half of the population is parasitemic), transmission is usually constant, and disease manifestations are moderated by the development of immunity. Mortality is largely restricted to infants and to nonimmune adults who migrate into the region. When the prevalence of disease is lower, transmission is typically intermittent. In this situation, solid immunity does not develop and the population suffers repeated, often seasonal, epidemics, the impact of which is shared by people of all ages.

Malaria kills 1–3 million annually; mostly children

Imported malaria may develop months after travel

Presently, it is estimated that 2 billion people live in malaria endemic areas in 103 of the poorest countries of Africa, Asia, Latin America, and Oceania (Fig 52–3). Between 25 and 50% of these persons are thought to be carrying the malaria parasite at any given time. From 1 to 3 million individuals, primarily African children, die of this disease annually. A recent study concluded that the development of resistance to chloroquine, the single most widely used antimalarial agent, has increased mortality four- to eightfold. Although endemic malaria disappeared from the United States three decades ago,

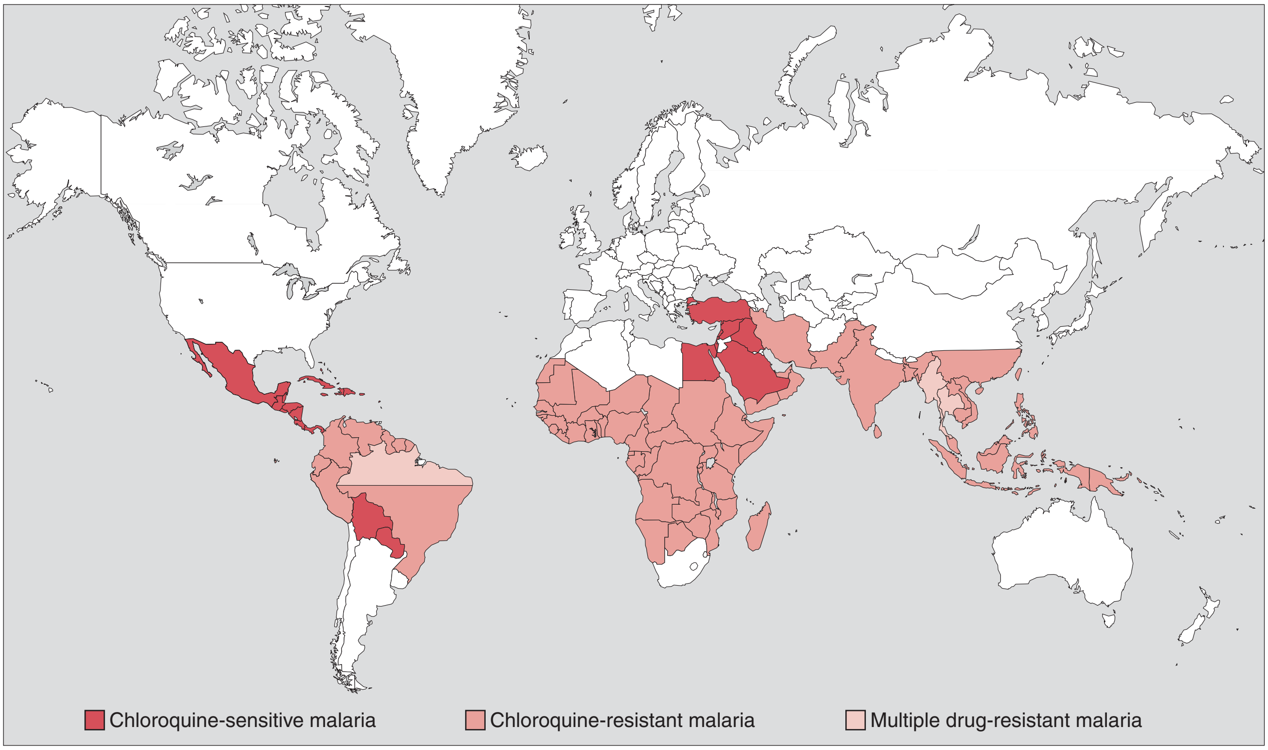



FIGURE 52–3

Distribution of malaria and drug-resistant *Plasmodium falciparum,* 1995. (*Data from the Centers for Disease Control and Prevention, Atlanta, Ga. From Mandell GL, Bennett JE, Dolin R* (*eds*). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, New York: Churchill Livingstone, 2002.*)

imported cases continue to be reported, and the recent worldwide resurgence of malaria combined with an increase in international travel has resulted in an increase in the number of US cases to approximately 1000 annually. Forty-five percent of patients with imported malaria have acquired the disease in Africa, 30% in Asia, and 10% in the Caribbean or Latin America. Fifty percent of recent infections have involved American travelers: nearly 60% of these acquired their infection in Africa. Clinical manifestations typically develop within 6 months of arrival of cases in the United States; however, one fourth of cases caused by *P. vivax* are delayed beyond that time. Approximately 40% of imported cases and almost all associated fatalities have been caused by the virulent *P. falciparum*. Tragically, most of these cases could have been prevented or successfully treated. Congenital malaria in infants born in the United States of mothers from malarious areas is occasionally observed. Infections transmitted by transfusions of whole blood, leukocytes, or platelets, or by organ transplantation are, fortunately, now unusual in this country due to the improved screening procedures of blood banks. Anopheline mosquitoes capable of transmitting malaria are present in the United States, and, rarely, malaria is transmitted from an imported case to individuals who have never traveled outside of the country.

## PATHOGENESIS

The fever, anemia, circulatory changes, and immunopathologic phenomena characteristic of malaria are all the result of erythrocytic invasion by the plasmodia.

## Fever

Fever, the hallmark of malaria, appears to be initiated by the process of RBC rupture that leads to the liberation of a new generation of merozoites (sporulation). To date, all attempts to detect the factor(s) mediating the fever have been unsuccessful. It is possible that parasite-derived pyrogens are released at the time of sporulation; alternatively, the fever might result from the release of interleukin-1 (IL-1) and/or tumor necrosis factor (TNF) from macrophages involved in the ingestion of parasitic or erythrocytic debris. Early in malaria, RBCs appear to be infected with malarial parasites at several different stages of development, each inducing sporulation at a different time. The resulting fever is irregular and hectic. Because temperatures in excess of 40°C destroy mature parasites, a single population eventually emerges, sporulation is synchronized, and fever occurs in distinct paroxysms at 48-hour or, in the case of *P. malariae,* 72-hour intervals. Periodicity is seldom seen in patients who are rapidly diagnosed and treated.

Fever associated with RBC rupture

Synchronization of sporulation causes cyclic fever

## Anemia

Parasitized erythrocytes are phagocytosed by a stimulated reticuloendothelial system or are destroyed at the time of sporulation. At times, the anemia is disproportionate to the degree of parasitism. Depression of marrow function, sequestration of erythrocytes within the enlarging spleen, and accelerated clearance of nonparasitized cells all appear to contribute to the anemia. The mechanisms responsible for the latter are unclear. Intravascular hemolysis, although uncommon, may occur, particularly in falciparum malaria. When hemolysis is massive, hemoglobinuria develops, resulting in the production of dark urine. This process in conjunction with malaria is known as **blackwater fever.**

Destruction of normal and parasitized RBCs causes anemia

Massive intravascular hemolysis can occur

## Circulatory Changes

The high fever results in significant vasodilatation. In falciparum malaria, vasodilatation leads to a decrease in the effective circulating blood volume and hypotension, which may be aggravated by other changes in the small vessels and capillaries. The intense parasitemias *P. falciparum* is capable of producing and the adhesion of infected RBCs to the endothelium of visceral capillaries can impair the microcirculation and precipitate tissue hypoxia, lactic acidosis, and hypoglycemia. Although all deep tissues are involved, the brain is the most intensely affected.

Blood flow decreased to vital organs

## Cytokines

Elevated levels of IL-1 and TNF are consistently found in patients with malaria. Probably released at the time of sporulation, these proteins are certainly an essential part of the host's immune response to malaria (see below). By modulating the effects of endothelial cells, macrophages, monocytes, and neutrophils, they may play an important role in the destruction of the invading parasite. However, TNF levels increase with parasite density and high concentrations appear harmful. TNF has been shown to cause upregulation of endothelial adhesion molecules; high concentrations might precipitate cerebral malaria by increasing the sequestration of *P. falciparum*–parasitized erythrocytes in the cerebral vascular endothelium. Alternatively, excessive TNF levels might precipitate cerebral malaria by directly inducing hypoglycemia and lactic acidosis.

Elevated cytokine levels contribute to injury

## Other Pathogenic Phenomena

Thrombocytopenia is common in malaria and appears to be related to both splenic pooling and a shortened platelet lifespan. Both direct parasitic invasion and immune mechanisms may be responsible. There may be an acute transient glomerulonephritis in falciparum malaria and progressive renal disease in chronic *P. malariae* malaria. These phenomena probably result from the host immune response, with deposition of immune complexes in the glomeruli.

Thrombocytopenia and nephritis common

## IMMUNITY

Initial immune response limits parasite multiplication but does not eliminate infection (premunition)

Once infected, the host quickly mounts a species- and strain-specific immunologic response that typically limits parasite multiplication and moderates the clinical manifestations of disease, without eliminating the infection—a phenomenon referred to as **premunition.** A prolonged recovery period marked by recurrent exacerbations in both symptoms and number of erythrocytic parasites follows. With time, these recrudescences become less severe and less frequent, eventually stopping altogether.

Antibody-mediated immunity important

The exact mechanisms involved in this recovery are uncertain. In simian and probably in human malaria, recovery is known to require the presence of both T and B lymphocytes. It is probable that the T lymphocytes act partially through their helper effect on antibody production. Some authorities have suggested that they also play a direct role through lymphokine production by stimulating effector cells to release nonspecific factors capable of inhibiting intraerythrocytic multiplication. The B lymphocytes begin production of stage- and strain-specific antiplasmodial antibodies within the first 2 weeks of parasitemia. With the achievement of high levels of antibodies, the number of circulating parasites decreases. The infrequency with which malaria occurs in young infants has been attributed to the transplacental passage of such antibodies. It is uncertain whether they are directly lethal, act as opsonizing agents, or block merozoite invasion of RBCs.

Antigenic variation could play a role in persistence

Suppressor T lymphocytes in spleen may protect parasites

In simian malaria, the parasite can undergo antigenic variation and thereby escape the suppressive effect of the antibodies. This antigenic variation leads to cycles of recrudescent parasitemia but ultimately to production of specific antibodies to the variants, and cure. It seems probable that similar changes occur in humans, leading to the eventual disappearance of erythrocytic parasites. With *P. falciparum* and *P. malariae,* which have no persistent hepatic forms, this results in cure. With *P. falciparum,* the disease typically does not exceed 1 year, but with *P. malariae* the erythrocytic infection can be extremely persistent, lasting in one case up to 53 years. How erythrocytic parasites circulating in numbers too small to be detected on routine blood films escape immunologic destruction remains a puzzle. In a closely related simian malaria, splenectomy results in rapid cure, suggesting that suppressor T lymphocytes in the spleen may play a protective role. In infection with *P. vivax* and *P. ovale,* latent hepatic infection may result in the discharge of fresh merozoites into the bloodstream after the disappearance of erythrocytic forms. This phenomenon, known as relapse, is capable of maintaining infection for 3 to 5 years.

# MALARIA: CLINICAL ASPECTS

## MANIFESTATIONS

Incubation period prolonged by suppressant use

The incubation period between the bite of the mosquito and the onset of disease is approximately 2 weeks. With *P. malariae* and with strains of *P. vivax* in temperate climates, however, this period is often more prolonged. Individuals who contract malaria while taking antimalarial suppressants may not experience illness for many months. In the United States, the interval between entry into the country and onset of disease exceeds 1 month in 25% of *P. falciparum* infections and 6 months in a similar proportion of *P. vivax* cases.

Malarial paroxysm: cold, hot, wet stages

The clinical manifestations vary with the species of plasmodia but typically include chills, fever, splenomegaly, and anemia. The hallmark of disease is the malarial paroxysm. This manifestation begins with a cold stage, which persists for 20 to 60 minutes. During this time, the patient experiences continuous rigors and feels cold. With the consequent increase in body temperature, the rigors cease and vasodilatation commences, ushering in a hot stage. The temperature continues to rise for 3 to 8 hours, reaching a maximum of 40 to 41.7°C before it begins to fall. The wet stage consists of a decrease in fever and profuse sweating. It leaves the patient exhausted but otherwise well until the onset of the next paroxysm.

Typical paroxysms first appear in the second or third week of fever, when parasite sporulation becomes synchronized. In falciparum malaria, synchronization may never take place, and the fever may remain hectic and unpredictable. The first attack is often severe and may persist for weeks in the untreated patient. Eventually the paroxysms become less regular, less frequent, and less severe. Symptoms finally cease with the disappearance of the parasites from the blood.

Typical paroxysms after 2–3 weeks when sporulation is synchronized

In falciparum malaria, capillary blockage can lead to several serious complications. When the central nervous system is involved (cerebral malaria), the patient may develop delirium, convulsions, paralysis, coma, and rapid death. Acute pulmonary insufficiency frequently accompanies cerebral malaria, killing about 80% of those involved. When splanchnic capillaries are involved, the patient may experience vomiting, abdominal pain, and diarrhea with or without bloody stools. Jaundice and acute renal failure are also common in severe illness. These pernicious syndromes generally appear when the intensity of parasitemia exceeds 100,000 organisms per cubic millimeter of blood. Most deaths occur within 3 days.

Cerebral falciparum malaria often lethal

## DIAGNOSIS

Malarial parasites can be demonstrated in stained smears of the peripheral blood in virtually all symptomatic patients. Typically, capillary or venous blood is used to prepare both thin and thick smears, which are stained with Wright or Giemsa stain and examined for the presence of erythrocytic parasites. Thick smears, in which erythrocytes are lysed with water before staining, concentrate the parasites and allow detection of very mild parasitemia. Nonetheless, it may be necessary to obtain several specimens before parasites are seen. Artifacts are numerous in thick smears, and correct interpretation requires experience. The morphologic differences among the four species of plasmodia allow their speciation on the stained smear by the skilled observer.

Thick and thin blood smears detect parasites

A number of attempts have been made to improve on the standard thin and thick smear. One such procedure involves acridine orange staining of centrifuged parasites in quantitative buffy coat (QBC) tubes. Although it is expensive, requires a fluorescence microscope, and permits less reliable parasite speciation, its rapidity and ease of use make it attractive to laboratories that are only occasionally called on to identify patients with malaria. Simple, specific card antigen detection procedures are now available. The most widely used test, ParaSight F, detects a protein (HRP2) excreted by *P. falciparum* within minutes. The test can be performed under field conditions and has a sensitivity more than 95%. A second rapid test, OptiMAL, detects parasite lactate dehydrogenase, and, unlike ParaSight F, can distinguish between *P. falciparum* and *P. vivax*. Serologic tests for malaria are offered at a few large reference laboratories but are used primarily for epidemiologic purposes. They are occasionally helpful in speciation and detection of otherwise occult infections. The recently completed sequencing of the malaria genome will lead to newer diagnostic methods.

Acridine orange stains and other rapid detection methods available

## TREATMENT

The indications for treatment rest on two factors. The first is the infecting species of *Plasmodium,* and the second is the immune status of the afflicted patient. Falciparum malaria is potentially lethal in nonimmune individuals such as new immigrants or travelers to a malarious area and immunosuppressed indigenous individuals such as pregnant women. These individuals must be treated emergently.

The complete treatment of malaria requires the destruction of three parasitic forms: the erythrocytic schizont, the hepatic schizont, and the erythrocytic gametocyte. The first terminates the clinical attack, the second prevents relapse, and the third renders the patient noninfectious to *Anopheles* and thus breaks the cycle of transmission. Unfortunately, no single drug accomplishes all three goals. The present strategy of chemotherapy is shown in Table 52–2.

Need to destroy all forms of the parasite

### Termination of Acute Attack

Several agents can destroy asexual erythrocytic parasites. Chloroquine, a 4-aminoquinoline, has been the most commonly used. It acts by inhibiting the degradation of hemoglobin,

TABLE 52–2

## Chemotherapy of Malaria

| Stage of Parasite | Clinical Goal | Drug |
|---|---|---|
| Erythrocytic schizont | Treat clinical attack | |
| | All species | Chloroquine |
| | CRFM | Quinine, antifolates, sulfonamides, artemisinin (regionally dependent) |
| | Suppress clinical attack | |
| | All species | Chloroquine |
| | CRFM | Antifolates, sulfonamides (regionally dependent) |
| Erythrocytic gametocyte | Prevent transmission | |
| | Relapsing malaria | Chloroquine |
| | Falciparum malaria | Primaquine, artemisinin |
| Hepatic schizont | Radical cure | |
| | Relapsing malaria | Primaquine |
| | Falciparum malaria | None required |

*Abbreviation:* CRFM, chloroquine-resistant falciparum malaria.

thereby limiting the availability of amino acids necessary for growth. It has been suggested that the weak basic nature of chloroquine also acts to raise the pH of the food vacuoles of the parasite, inhibiting their acid proteases and effectiveness. When originally introduced, it was rapidly effective against all four species of plasmodia and, in the dosage used, free of serious side effects. However, chloroquine-resistant strains of *P. falciparum* are now widespread in Africa and Southeast Asia; they are also found, although less frequently, in other areas of Asia and in Central America and South America. Other schizonticidal agents include quinine/quinidine, antifolate–sulfonamide combinations, mefloquine, halofantrine, and the artemisinins. Except for the artemisinins, malaria resistance to all of the above agents is increasing. The artemisinins are also unique in their capacity to reduce transmission by preventing gametocyte development.

Chloroquine inhibits hemoglobin degradation by parasite

Artemisinins prevent gametocyte development

Strains of *P. malariae, P. ovale,* and *P. vivax* (except for some acquired in the South Pacific and South America) remain sensitive to chloroquine and may be treated with this agent. *P. vivax* infections acquired in New Guinea and Sumatra, however, should be assumed to be chloroquine-resistant and managed with mefloquine alone or in combination with other agents. *P. falciparum* has now become variably resistant to all drug groups except the artemisinin compounds (see Fig 52–3).

Resistance of chloroquine and other drugs now common with *P. falciparum*

There is a growing consensus that the most effective way to slow the further development of drug-resistant strains of *P. falciparum* is to use one of the artemisinins in combination with quinine/quinidine, antifolate–sulfonamide compounds, mefloquine, or halofantrine.

Combination therapy may be necessary

## Radical Cure

In *P. vivax* and *P. ovale* infections, hepatic schizonts persist and must be destroyed to prevent reseeding of circulating erythrocytes with consequent relapse. Primaquine, an 8-aminoquinaline, is used for this purpose. Some *P. vivax* infections acquired in Southeast Asia and New Guinea fail initial therapy due to relative resistance to this 8-aminoquinaline. Retreatment with a larger dose of primaquine is usually successful. Unfortunately, primaquine may induce hemolysis in patients with G6PD deficiency. Persons of Asian, African, and Mediterranean ancestry should thus be screened for this abnormality before treatment. Chloroquine destroys the gametocytes of *P. vivax, P. ovale,* and *P.*

Primaquine used to destroy hepatic schizonts of *P. vivax* and *P. ovale* *malariae* but not those of *P. falciparum.* Primaquine and artemisinins, however, are effective for this latter species.

# PREVENTION

## Personal Protection

In endemic areas, mosquito contact can be minimized with the use of house screens, insecticide bombs within rooms, and/or insecticide-impregnated mosquito netting around beds. Those who must be outside from dusk to dawn, the period of mosquito feeding, should apply insect repellent and wear clothing with long sleeves and pants. In addition, it is possible to suppress clinical manifestations of infection, should they occur, with a weekly dose of chloroquine. In areas where chloroquine-resistant strains are common, an alternative schizonticidal agent should be used. Mefloquine or doxycycline are usually preferred. The antifolate pyrimethamine plus a sulfonamide can be taken as well. However, use of this combination is occasionally accompanied by serious side effects, so it is recommended only when mefloquine- and doxycycline-resistant strains are present in the area, and then only for individuals residing in areas of intense transmission for prolonged periods of time. On leaving an endemic area, it is necessary to eradicate residual hepatic parasites with primaquine before discontinuing suppressive therapy.

Mosquito protection with screens and repellents

Chemoprophylaxis choice must consider resistance in area

## General

Malaria control measures have been directed toward reducing the infected human and mosquito populations to below the critical level necessary for sustained transmission of disease. The techniques employed include those mentioned previously, treatment of febrile patients with effective antimalarial agents, chemical or physical disruption of mosquito breeding areas, and use of residual insecticide sprays. An active international cooperative program aimed at the eradication of malaria resulted in a dramatic decline in the incidence of the disease between 1956 and 1968. Eradication was not achieved, however, because mosquitoes became resistant to some of the chemical agents used, and today malaria still infects 200 to 300 million inhabitants of Africa, Latin America, and Asia. Tropical Africa alone accounts for 100 million of the afflicted and for most of the 1 to 3 million deaths that occur annually as a result of this disease. The long-term hope for progress in these areas now depends on the development of new technologies.

Reduce human reservoir and eradicate mosquitoes

Attempts at eradication have failed

## Vaccines

Three advances in the last decade have produced the hope that an effective malaria vaccine might be within reach of medical science for the first time. The establishment of a continuous in vitro culture system provided the large quantities of parasite needed for antigenic analysis. Development of the hybridoma technique allowed the preparation of monoclonal antibodies with which antigens responsible for the induction of protective immunity could be identified. Finally, recombinant DNA procedures enabled scientists to clone and sequence the genes encoding such antigens, permitting the amino acid structure to be determined and peptide sequences suitable for vaccine development to be identified.

Peptide sequences for vaccine development being identified

As immunity to malaria is stage specific, the relative advantages and disadvantages of vaccines prepared against each of the plasmodial stages found in the human host (sporozoite, merozoite, and gametocyte) need to be considered. An effective sporozoite vaccine, by blocking the invasion of hepatocytes by mosquito-introduced sporozoites, would prevent the establishment of the infection within the host and, if widely administered, would interrupt parasite transmission within a community. However, to be effective, a sporozoite vaccine would have to prevent the invasion of all injected sporozoites. Theoretically, if even a single parasite reached and penetrated a liver cell, it would multiply intracellularly and later enter the bloodstream to invade erythrocytes. The patient could develop clinical disease and serve as a reservoir for subsequent transmission to others. A vaccine directed at the erythrocytic or merozoite stage, although preventing neither hepatic nor bloodstream infection, would limit the severity of the parasitemia

Sporozoite vaccine could help prevent initiation of infection

Combination vaccines may be necessary

and thus moderate or abort clinical manifestations of disease. Gametogenesis, and thus parasite transmission, would probably proceed unimpaired. Antibodies formed in response to a gametocyte vaccine might block the union of male and female gametes within the mosquito gut, interrupting parasite transmission. It would, however, neither prevent nor moderate malaria in the immunized patient. The limitations of each vaccine type has led some investigators to advocate the combination of all three in a single polyvalent preparation. Unfortunately, the results of field tests of a number of candidate vaccines have been disappointing.

# TOXOPLASMA GONDII

## PARASITOLOGY

Asexual and sexual cycles in felines

Three forms of human disease

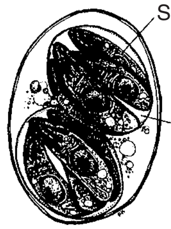
Like the plasmodia, *Toxoplasma gondii,* the cause of toxoplasmosis, is an obligate intracellular sporozoan. It differs from *Plasmodium* in that both sexual and asexual reproductive cycles occur within the gastrointestinal tract of felines, the definitive host. The disease is transmitted to other host species by the ingestion of oocysts passed in the feces of infected felines.

### MORPHOLOGY

Spread to humans from felines via fecal–oral route

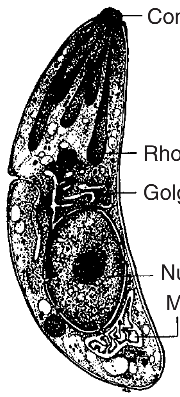
Tissue cysts killed by cooking as well as freezing and thawing

*T. gondii* was first demonstrated in 1908 in the gondi, an African rodent, by Nicolle and Marceaux. Its name, derived from the Greek toxo (arc), is based on the characteristic shape of the organism. All strains of this parasite appear to be closely related antigenically. The major morphologic forms of the parasite are the oocyst, trophozoite, and tissue cyst.




*Toxoplasma* oocyst

10 µm




*Toxoplasma* tachyzoite (trophozoite)

3 µm

#### Oocyst

The oocyst is ovoid, measures 10 to 12 $\mu$m in diameter, and possesses a thick wall that makes it resistant to most environmental challenges. It may be destroyed by heat in excess of 66°C and chemicals such as iodine and formalin. In its immature form, the center of the cyst lacks internal structure. With maturation two sporocysts appear, and later four sporozoites may be discerned within each sporocyst. Sporulation does not occur at temperatures below 4°C or above 37°C. This form is responsible for the spread of the parasites from felines to other warm-blooded animals via the fecal–oral route.

#### Tachyzoite (Trophozoite)

The term "trophozoite" is used in its broadest sense to refer to the asexual proliferative forms responsible for cell invasion and clinical disease. In different stages of the asexual cycle it is referred to by several other terms, including merozoite and tachyzoite. It is crescent or arc shaped, measures 3 by 7 $\mu$m, and can invade all nucleated cell types. Although tachyzoites are obligate intracellular organisms, they may survive extracellularly in a variety of body fluids for periods of hours to days. They cannot, however, survive the digestive activity of the stomach and therefore are not infective on ingestion.

#### Tissue Cysts

Cysts measure 10 to 200 $\mu$m in diameter. The contained organisms, referred to as bradyzoites, are similar to tachyzoites, but are smaller and divide more slowly. Tissue cysts are resistant to digestive enzymes, and like oocysts, are infectious to the animal that ingests them. They survive normal refrigerator temperatures but are killed by freezing and thawing and by normal cooking temperatures.

## LIFE CYCLE

### Definitive Host

Infection in cat ileal cells

Fusion of gametes leads to oocyst formation; shed in feces

Sporulate in external environment

Sexual reproduction of *T. gondii* occurs only in the intestinal tract of felines, most importantly in the domestic cat. Ingested parasites enter the epithelial cells of the ileum by mechanisms that remain poorly defined. Intracellularly, the trophozoites reside within a membrane-bound vacuole and undergo schizogony. With cell rupture, merozoites are released. The merozoites infect adjacent epithelial cells; they then repeat another asexual cycle or eventually differentiate into gametocytes, initiating sexual reproduction. Fusion of the mature male and female gametes leads to the formation of an oval, thick-walled oocyst that is then shed in the feces. In the typical infection, millions of these structures are released daily for 1 to 3 weeks. The oocysts are immature at the time of shedding and must complete sporulation in the external environment. In this process, two sporocysts, each containing four sporozoites, develop within each oocyst. The time required for sporulation varies from 1 day to 3 weeks, depending on the ambient temperature and moisture. Once mature, the resistant oocysts may remain viable and infectious for many years in soil.

### Intermediate Hosts

Mature oocysts infect hosts orally

Released sporozoites invade macrophages

Cysts develop and can persist for life of host

After ingestion by a susceptible warm-blooded animal, sporozoites are released from the disrupted oocyst and enter macrophages. Within these cells, they are transported through the lymphohematogenous system to all organ systems. Continued intracellular schizogony results in macrophage rupture and release of new parasites, which may invade any adjacent nucleated host cell and continue the asexual cycle. With the development of host immunity, many of the parasites are destroyed. Within the cells of certain organs, particularly the brain, heart, and skeletal muscle, the trophozoites produce a membrane that surrounds and protects them: within this tissue cyst, multiplication continues at a more leisurely pace. Eventually, cysts that measure up to 200 $\mu$m in diameter and contain more than a thousand organisms are produced. These cysts persist intact for the life of the host or rupture, producing parasitologic relapse. If they are ingested by a carnivore, they survive the digestive enzymes and initiate infection in the new host.

## TOXOPLASMOSIS

CLINICAL CAPSULE

*Toxoplasma* can infect most warm-blooded animals, both domestic and wild; it is thus the most cosmopolitan of parasites. Approximately 50% of the human population of the United States has been infected. In the overwhelming majority of persons, infection is chronic, asymptomatic, and self-limiting. Clinical disease presents in three major forms: (1) self-limiting febrile lymphadenopathy, (2) highly lethal infection of immunocompromised patients, and (3) congenital infection of infants.

## EPIDEMIOLOGY

### Prevalence and Distribution

Worldwide distribution among mammals and birds

Toxoplasmosis is a cosmopolitan disease that occurs in almost all mammals and many birds. Human infections are found in every region of the globe; in general, the incidence is higher in the tropics and lower in cold and/or arid regions. In the United States, the prevalence of positive serologic evidence for the disease increases with age. By adulthood, approximately 50% of Americans can be shown to have circulating antibodies against *T. gondii.*

### Transmission

Although it is known that humans may acquire toxoplasmosis in a variety of ways, data on their relative frequency are both meager and conflicting. It is likely that the route of transmission varies from population to population, and perhaps from age to age, within any given area. The most important transmission mechanisms are discussed below.

### *Ingestion of Oocysts*

Increased hazard to children by close contact with contaminated areas

Felinophobes are inclined to the view that the deposition of oocysts in the feces of cats and their subsequent ingestion by the unsuspecting owner is the most frequent way in which humans acquire this important infection. Disease epidemics associated with exposure to infected cats have been reported. Unfortunately, data from studies relating the frequency of feline exposure to the prevalence of positive serologic tests are conflicting. Acutely infected cats shed oocysts for only a few weeks. It has been shown, however, that chronically infected felines can occasionally reshed oocysts, and prevalence studies have demonstrated that 1% of domestic cats excrete oocysts at any given time. The large number of these structures passed during active shedding and their prolonged survival in the external environment greatly enhance their chance of transmission. Particularly at risk are individuals such as children at play, who may come in close contact with areas likely to be contaminated with cat feces, and adults responsible for changing a cat's litter box. It is also possible that insects can mechanically transfer oocysts to human food.

### *Ingestion of Tissue Cysts*

Cysts present in meat

Tissue cysts have been frequently demonstrated in meat produced for human consumption. They are most common in pork (25%) and mutton (10%) and less so in beef and chicken (<1%). Although such cysts are killed at normal (well-done) cooking temperatures, an impressive array of epidemiologic information links the handling and/or ingestion of raw or undercooked meat with serologic and, occasionally, clinical evidence of disease. Confounding these data is an Indian study that demonstrated no difference between meat eaters and vegetarians in the incidence of positive serologic tests.

### *Congenital*

Transplacental transmission highest in third trimester

Approximately 1 of every 500 pregnant women acquires acute toxoplasmosis, and approximately 10 to 20% of the involved women become symptomatic. Regardless of the clinical status of the infected mother, the parasite involves the fetus in 33 to 50% of all acute maternal infections. The risk of transplacental transmission is independent of the clinical severity of the disease in the mother, but does correlate with the stage of gestation at which she is exposed. Fetal involvement occurs in 17% of first-trimester and 65% of third-trimester infections. Conversely, the earlier a fetal infection is acquired, the more severe it is likely to be. Overall, 20% of fetuses experienced severe consequences; a similar proportion develop mild disease. The remainder are asymptomatic.

### *Miscellaneous*

Transmitted by transfusions and organ transplants

In addition to causing congenital infection, trophozoites have been responsible for disease transmission in a number of other situations, including laboratory accidents, transfusions of whole blood and leukocytes, and organ transplantation. Because trophozoites may survive for several hours in body fluids or exudates of acutely infected humans, it is possible for infection to occur after contact with such materials.

## PATHOGENESIS AND IMMUNITY

Dissemination in immunosuppressed subjects

In the primary infection, the proliferation of trophozoites results in the death of involved host cells, stimulation of a mononuclear inflammatory reaction, and a parasite-specific secretory IgA response. In immunodeficient hosts, rapid organism proliferation continues, producing numerous widespread foci of tissue necrosis. The consequences are most serious in organs such as the brain, where the potential for cell regeneration is limited.

In normal hosts, however, acute infection is rapidly controlled with the development of humoral and cellular immunity. Extracellular parasites are destroyed, intracellular multiplication is hindered, and tissue cysts are formed. With the exception of lysis of extracellular parasites by antibody and complement, cell-mediated immunity appears to play the principal role in this process, mediated in part by IL-2, interferon-$\alpha$, and cytotoxic T cells. Immunity appears to be lifelong, possibly because of survival of the parasite in the tissue cysts. The cysts, which are found most frequently in the brain, retina, heart and skeletal muscle, normally produce little or no tissue reaction. The suppression of cell-mediated immunity that accompanies serious illness, or the administration of immunosuppressive agents, may lead to the rupture of a cyst and the release of trophozoites. Their subsequent proliferation and the intense antibody reaction to their presence results in an acute exacerbation of the disease.

Immunity is primarily cell-mediated

# TOXOPLASMOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

In the vast majority of patients, infection with *T. gondii* is completely asymptomatic. Clinical manifestations, when they do appear, vary with the type of host involved. In general, they may be grouped into one of the three syndromes listed below.

### Congenital Toxoplasmosis

Immune mechanisms are poorly developed in utero. As a result, a large proportion of fetal infections results in clinical illness. If the infection spreads to the central nervous system, the outcome is often catastrophic. Abortion and stillbirth are the most serious consequences. Liveborn children may demonstrate microcephaly, hydrocephaly, cerebral calcifications, convulsions, and psychomotor retardation. Disease of this severity is usually accompanied by evidence of visceral involvement, including fever, hepatitis, pneumonia, and skin rash. Infants infected later in prenatal development demonstrate milder disease. Many appear healthy at birth but develop epilepsy, retardation, or strabismus months or years later. Probably the most common delayed manifestation of congenital toxoplasmosis is chorioretinitis. This condition, which is thought to result from the reactivation of latent tissue cysts, typically presents during the second or third decade of life as recurrent bouts of eye pain and loss of visual acuity. The lesions are usually bilateral but focal. If the retinal macula is not involved, vision improves as the inflammation subsides. *T. gondii* accounts for 25% of all cases of granulomatous uveitis seen in the United States.

Infection in utero can produce malformations, chorioretinitis, and stillbirth

### Normal Host

The most common clinical manifestation of toxoplasmosis acquired after birth is asymptomatic localized lymphadenopathy. The cervical nodes are most frequently involved, but nontender enlargement of other regional groups, including the retroperitoneal nodes, also occurs. At times, the adenopathy is accompanied by fever, sore throat, rash, hepatosplenomegaly, and atypical lymphocytosis, thus mimicking the clinical and laboratory manifestations of infectious mononucleosis. Occasionally the normal host develops severe visceral involvement, which may be manifested as meningoencephalitis, pneumonitis, myocarditis, or hepatitis. Chorioretinitis following postnatally acquired infection, although documented, is uncommon. Unlike congenitally acquired ocular disease, it occurs during midlife and is generally unilateral.

Fever and lymphadenopathy can mimic infectious mononucleosis

### Immunocompromised Host

In the immunocompromised host, toxoplasmosis is a serious, often fatal disease. If primary infection is acquired while a patient is undergoing immunosuppressive therapy for malignancy or organ transplantation, widespread dissemination of the infection with necrotizing pneumonitis, myocarditis, and encephalitis may occur. More commonly, acute disease in this population results from the activation of chronic, latent infection by immunosuppressive therapy, or the acquisition of a concurrent immunosuppressive infection, particularly acquired immunodeficiency syndrome (AIDS). Encephalitis occurs in 50% of such cases and in more than 90% of fatal cases. Toxoplasmic encephalitis is particularly common in AIDS patients; it is seen in approximately 10% of those with circulating toxoplasma antibodies. As such, it is a major cause of morbidity and mortality in this patient population. Clinically, encephalitis may present as a meningoencephalitis, diffuse encephalopathy, or mass lesion.

Primary infection or reactivation of latent infections can produce severe, widespread disease

AIDS patients develop encephalitis

## DIAGNOSIS

The diagnosis may be established by a variety of methods. In acute toxoplasmic lymphadenitis, the histologic appearance of the involved nodes is often pathognomonic. The trophozoite may be demonstrated in tissue with Wright or Giemsa stain. Electron microscopy and indirect fluorescent antibody techniques have also been used successfully on heart transplant or brain tissue obtained by biopsy. Although tissue cysts are selectively stained by periodic acid–Schiff, their presence is not indicative of acute disease. Isolation of the organism can be accomplished by inoculating blood or other body fluids into mice or tissue cultures. Inoculation of other tissues is not usually helpful, because a positive result may only reflect the presence of latent tissue cysts.

Demonstration of parasite in histopathologic specimens

Serologic procedures are the primary method of diagnosis. To establish the presence of acute infection, it is usual to demonstrate a fourfold rise in the IgG antibody titer between acute and convalescent serum specimens. Peak titers are often reached within 4 to 8 weeks, so the acute serum must be collected early in the course of illness. Of the many tests developed for the detection of IgG antibodies, the indirect hemagglutination test and the indirect fluorescent antibody test are those most frequently used; they both are sensitive and highly specific. With these tests, titers of 1:1000 or more are usually detected after an acute infection. These levels gradually fall but may remain high for many years.

Serodiagnosis is the primary approach

The detection of IgM antibodies provides a more rapid confirmation of acute infection. As detected by an indirect fluorescent antibody technique, these antibodies appear within the first week of infection, peak in 2 to 4 weeks, and quickly revert to negative. It also appears that IgM antibodies are produced after reactivation of latent disease. A single high titer (1:80 or more) therefore establishes the presence of acute infection or reactivation. Unfortunately, this test has been difficult to standardize, lacks sensitivity in neonates and immunocompromised (particularly AIDS) hosts, and is not widely available. Recently introduced enzyme immunoassays (EIA) for IgM antibody circumvent many of these difficulties, but still produce some false-positive results, and are not sufficiently sensitive in AIDS patients. A modification of the EIA procedure, the antibody-capture enzyme immunoassay, significantly improves both the sensitivity and specificity of the original procedure. Examination of urine and other body fluids for the presence of toxoplasma antigen, or DNA by the polymerase chain reaction, have been shown to be useful adjunctive tests in immunocompromised individuals; currently these procedures are not generally available to clinical laboratories.

Rising titers of IgG or detection of IgM suggest acute infection or reactivation

## TREATMENT AND PREVENTION

Usually, patients do not require therapy unless symptoms are particularly severe and persistent or unless vital organs, such as the eye, are involved. Immunocompromised and pregnant women, however, should be treated if acute infection (or reactivation) is documented (Table 52–3). Routine serial serologic testing of such individuals would allow early detection of infected patients and enhance the prospects of a successful outcome. It is now clear that early treatment of acutely infected pregnant women significantly reduces the incidence of severe congenital infections and reduces the ratio of benign to subclinical forms in infants. At present, the most commonly used therapeutic regimen in the United States is the combination of pyrimethamine and sulfonamides. Unfortunately, the former

Spiramycin used to prevent congenital infection

TABLE 52–3

**Indications for Treatment of Toxoplasmosis**[a]

| Serologic Criteria | Clinical Criteria |
|---|---|
| Elevated IgM titers | Potential laboratory acquired infection |
| Fourfold rise in IgG titers | Pregnant woman |
| Very high IgG titers (>1:1000) | Neonate |
| | Immunocompromised patient (including AIDS) |
| | Severe constitutional symptoms |
| | Vital organ involvement (including active chorioretinitis) |

*Abbreviation:* Ig, immunoglobulin.

[a]Must satisfy one serologic plus one clinical criterion.

drug is teratogenic and should not be used in the first trimester of pregnancy; spiramycin, a cytostatic macrolide, is often substituted in this setting.

Although the pyrimethamine–sulfonamide combination is very effective against tachyzoites, it is inactive against the cyst forms. As both parasitic forms are present in patients with toxoplasmic encephalitis, recrudescence of illness generally follows completion of standard therapy in AIDS patients. This may be prevented by initiating chronic, low-dose suppressive therapy following the completion of the standard regimen. Atovaquone, a recently introduced hydroxynaphthoquinone, possesses activity against both tachyzoites and cysts. Its use, therefore, may result in radical cure of toxoplasma encephalitis, eliminating the need for chronic suppression.

Atovaquone is active against tachyzoites and cysts

Prevention should be directed primarily at pregnant women and immunologically compromised hosts. Hands should be carefully washed after handling uncooked meat. Cysts in meat can be destroyed by proper cooking (56°C for 15 min) or by freezing to –20°C. Cat feces should be avoided, particularly the changing of litter boxes.

## CRYPTOSPORIDIA

Cryptosporidia (“hidden-spore”) are small parasites that can infect the intestinal tract of a wide range of mammals, including humans. Like other sporozoan parasites, they are obligate intracellular organisms that exhibit alternating cycles of sexual and asexual reproduction. As with *Toxoplasma,* both cycles are completed within the gastrointestinal tract of a single host. Long recognized as an important cause of diarrhea in animals, cryptosporidia were not identified as causes of human enteritis until 1976.

## PARASITOLOGY

### MORPHOLOGY

Regardless of animal host, all strains of this tiny (2 to 6 $\mu$m) parasite appear morphologically identical. Although all strains can reasonably be regarded as a single species, the one that infects humans and cattle is often referred to as *C. parvum*. The organisms appear as small spherical structures arranged in rows along the microvilli of the epithelial cells. They are readily stained with Giemsa and hematoxylin–eosin. Although they

Small spherical particles associated with microvilli

Oocysts are acid-fast

remain external to the cytoplasm of the intestinal epithelial cell, they are covered by a double membrane derived from the reflection, fusion, and attenuation of the microvilli, and are thus, by definition, intracellular organisms. Oocysts shed into the intestinal lumen mature to contain four sporozoites; their cell wall provides the unusual property of acid fastness, allowing them to be visualized with stains generally employed for mycobacteria.

## LIFE CYCLE

Mature, infective oocysts excreted in stools

Infective oocysts are excreted in the stool of the parasitized animal. Unlike those of *Toxoplasma,* cryptosporidia oocysts are fully mature and immediately infective on passage in the feces. Following ingestion by another animal, sporozoites are released from the oocyst and attach to the microvilli of the small bowel epithelial cells, where they are transformed into trophozoites. These divide asexually by multiple fission (schizogony) to form schizonts containing eight daughter cells known as type 1 merozoites. On release from the schizont, each daughter cell attaches itself to another epithelial cell, where it repeats the schizogony cycle, producing another generation of type 1 merozoites.

Protective cell wall ensures survival of oocysts

Eventually, schizonts containing four type 2 merozoites are seen. Incapable of continued asexual reproduction, these develop into male (microgamete) and female (macrogamete) sexual forms. Following fertilization, the resulting zygote develops into an oocyst that is shed into the lumen of the bowel. The majority possess a thick protective cell wall that ensures their intact passage in the feces and survival in the external environment.

Some thin-walled oocysts can autoinfect

Approximately 20% fail to develop the thick protective wall. The cell membrane ruptures, releasing infective sporozoites directly into the intestinal lumen and initiating a new "autoinfective" cycle within the original host. In the normal host, the presence of innate or acquired immunity dampens both the cyclic production of type 1 merozoites and the formation of thin-walled oocysts, halting further parasite multiplication and terminating the acute infection. In the immunocompromised, both presumably continue, explaining why such individuals develop severe, persistent infections in the absence of external reinfection.

# CRYPTOSPORIDIOSIS

CLINICAL CAPSULE

Cryptosporidiosis is an intestinal illness acquired from domestic animals. The course includes profuse watery diarrhea, vomiting, and weight loss. Spontaneous complete recovery is the usual outcome.

## EPIDEMIOLOGY

Animal reservoirs and person-to-person transmission both important

Cryptosporidiosis appears to involve most vertebrate groups. In all species, infection rates are highest among the young and immature. Experimental and epidemiologic data suggest that domestic animals constitute an important reservoir of disease in humans. However, outbreaks of human disease in day-care centers, hospitals, and urban family groups indicate that most human infections result from person-to-person transmission. In Western countries, between 1 and 4% of small children presenting to medical centers with gastroenteritis have been shown to harbor cryptosporidia oocysts. In third world countries, the rates have varied from 4 to 11%. In some outbreaks of diarrhea in day-care centers, the majority of attendees were found to have oocysts in their stool.

Infection rates highest in young children

Infection rates in adults suffering from gastroenteritis is approximately one third of that reported in children; it has been highest in family members of infected children, medical personnel caring for patients with cryptosporidiosis, male homosexuals, and travelers to foreign countries. In the United States, the parasite has been identified in 15% of patients with AIDS and diarrhea; in Haiti and Africa, 50% of such individuals may be involved. Asymptomatic carriage is uncommon. Other enteric pathogens, particularly *Giardia lamblia,* are recovered from a significant minority of infected patients.

Because oocysts are found almost exclusively in stool, the principal transmission route is undoubtedly by direct fecal–oral spread. Transmission via contaminated water has been documented, and the hardy nature of the oocysts makes it likely that there is also indirect transmission via contaminated food and fomites.

Can be transmitted via contaminated water

## PATHOGENESIS AND IMMUNITY

Although the jejunum is most heavily involved, cryptosporidia have been found throughout the gastrointestinal tract, particularly in immunocompromised subjects. Cryptosporidial cholecystitis is seen with some frequency in AIDS patients with enteritis. By light microscopy, bowel changes appear minimal, consisting of mild to moderate villous atrophy, crypt enlargement, and a mononuclear infiltrate of the lamina propria. The pathophysiology of the diarrhea is unknown, but its nature and intensity suggest that a cholera-like enterotoxin may be involved. The vital role played by the host's immune status in the pathogenesis of the disease is indicated by both the enhanced susceptibility of the young to infection and the prolonged severe clinical disease seen in immunocompromised patients. Indirect evidence suggests antibodies in the intestinal lumen exert a protective effect against initial *C. parvum* infection. Experimental animal studies indicate that CD4+ T lymphocytes and interferon play independent roles in the immunologic clearance of the parasite.

Minimal intestinal pathology

Prolonged disease in AIDS patients

# CRYPTOSPORIDIOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

Immunocompetent patients usually note the onset of explosive, profuse, watery diarrhea 1 to 2 weeks after exposure. Typically, the illness persists for 5 to 11 days and then rapidly abates. Occasionally, purging, accompanied by a mild malabsorption and weight loss, continues for up to 1 month. A few patients complain of nausea, anorexia, vomiting, and low-grade fever. Except for its shorter duration, more prominent abdominal pain, and relative lack of flatulence, the clinical manifestations of cryptosporidiosis closely resemble those produced by *G. lamblia*. Radiographic and endoscopic examinations of the gut are either normal or demonstrate mild, nonspecific abnormalities. Recovery is complete, and neither relapse nor reinfection has been reported.

Self-limiting diarrhea in normal hosts

Cryptosporidiosis has been described in patients with a broad range of immunodeficiencies, including childhood malnutrition in third world countries, AIDS, and congenital hypogammaglobulinemia, and in those resulting from cancer chemotherapy and immunosuppressive management of organ transplants. In such patients, cryptosporidiosis is usually indolent in onset and manifestations are similar to those seen in normal hosts, but the diarrhea is more severe. Fluid losses of up to 25 L/day have been described. Patients with biliary cryptosporidiosis present with typical manifestations of cholecystitis and cholangitis. Unless the immunologic defect is reversed, the disease usually persists for the duration of the patient's life. Weight loss is often prominent. The prognosis depends on the nature of the underlying immunologic abnormality; half of patients with AIDS die within 6 months. Although other intercurrent infections are usually the direct cause of death, malnutrition and complications of parenteral nutrition contribute.

## DIAGNOSIS

The diagnosis of cryptosporidiosis is established by the recovery and identification of *Cryptosporidium* oocysts in a recently passed or preserved diarrheal stool. Oocyst excretion is most intense during the first week of illness, tapers during the second week, and generally stops with the cessation of diarrhea. Because cryptosporidia oocysts are one of

Detection of oocysts by acid-fast or immunofluorescent stains the few acid-fast particles found in feces, a definitive identification can be established with any one of the acid-fast staining procedures developed for mycobacteria. A direct immunofluorescence antibody stain using a monoclonal antibody to oocyst wall has been recently introduced that appears to be superior to acid-fast stains. When direct examinations are negative, concentration procedures are used and the concentrate restained. Immunofluorescence and EIAs for the detection of anticryptosporidial antibodies are now available.

## TREATMENT AND PREVENTION

Specific treatment remains problematic

In the immunocompetent patient, the disease is self-limited and attempts at specific antiparasitic therapy are not warranted; rehydration may be required in small children. In the immunocompromised host, the severity and chronicity of the diarrhea warrants therapeutic intervention. Unfortunately, there is no uniformly effective anticryptosporidial agent available at this time. Paromomycin, a luminal antimicrobic, has been shown to reduce the intensity of diarrhea in some patients, and parenteral octreotide acetate, a somatostatin analog, has been useful in decreasing stool volumes. The only uniformly successful approach has been the reversal of underlying immunologic abnormalities. When appropriate, withdrawal of cancer chemotherapy agents or immunosuppressive drugs may result in a cure.

The stools of patients with cryptosporidiosis are infectious. Stool precautions should be instituted at the time the diagnosis is first suspected; for the immunosuppressed patient, this should be whenever diarrhea, regardless of presumed etiology, is first noted. This is particularly important in cancer chemotherapy and transplantation units, where spread of the disease from a symptomatic patient to other immunosuppressed patients can have life-threatening consequences.

## ADDITIONAL READING

Bojang KA, et al. Efficacy of RTS,S/ASO2 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomized trial. *Lancet* 2001;358:1927–1934.

Curtis CF, Lines JD. Should DDT be banned by international treaty? *Parasitol Today* 2000;16:119–121.

Denkers DY, Gassinelli RT. Regulation and function of T-cell–mediated immunity during *Toxoplasma gondii* infection. *Clin Microbiol Rev* 1998;11:569–588.

Foulin W, et al. Treatment of toxoplasmosis during pregnancy. *Am J Obstet Gynecol* 1999;189:410.

Frenkel JK, Ruiz A. Endemicity of toxoplasmosis in Costa Rica. Transmission between cats, soil, intermediate hosts and humans. *Am J Epidemiol* 1981;113:254–269. This article is the most comprehensive study on the role of cats in the transmission of toxoplasmosis. It suggests that humans are infected primarily from soil contaminated with cat feces, rather than direct contact.

Griffiths JK. Human cryptosporidiosis: epidemiology, transmission, clinical disease, treatment and diagnosis. *Adv Parasitol* 1998;40:37.

Guerin PJ, et al. Malaria: current status of control, diagnosis, treatment, and a proposed agenda for research and development. *Lancet (Infect Dis)* 2002;2:564–573. This article brings clarity, comprehensiveness, and brevity to one of the two or three greatest infectious disease scourges of mankind. It is a “must read” for anyone intent on exploring this disease.

Heyworth MF. Immunology of *Giardia* and *Cryptosporidium* infections. *J Infect Dis* 1992;166:465–472. A review of the immunology of these two important protozoa.

Krogstad DJ. Malaria as a reemerging disease. *Epidemiol Rev* 1996;18:77–89.

Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. *Clin Infect Dis* 1995;15:211–222. A comprehensive review of an increasingly common presentation of toxoplasmosis.

Malaguamera L, Musumeci S. The immune response to *Plasmodium falciparum* malaria. *Lancet (Infectious Diseases)* 2002;2:472– 478. A concise review of recent developments.

Manabe YC, et al. Cryptosporidiosis in patients with AIDS: correlates of disease and survival. *Clin Infect Dis* 1998;27:536–542.

Newton P, White N. Malaria: new developments in treatment and prevention. *Annu Rev Med* 1999;50:179.

Phillips RS. Current status of malaria and potential for control. *Clin Microbiol Rev* 2001;14:208–226.

White NJ. Malaria pathophysiology. In *Malaria: Parasite Biology, Pathogenesis, Protection,* I Sherman (ed). Washington DC: ASM Press; 1998, pp 371–385.

Whitworth J, et al. Effect of HIV-1 and increasing suppression on malaria parasitology and clinical episodes in adults in rural Uganda: a cohort study. *Lancet* 2000;356: 1051–1056.

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CHAPTER 53

# Rhizopods

JAMES J. PLORDE

Rhizopods, or amebas, are the most primitive of the protozoa. They multiply by simple binary fission and move by means of cytoplasmic organelles called pseudopodia. These projections of the relatively solid ectoplasm are formed by streaming of the inner, more liquid endoplasm. They move the ameba forward and, incidentally, engulf and internalize food sources found in its path. Most amebas, when faced with a hostile environment, can produce a chitinous, external wall that surrounds and protects them. These forms are referred to as cysts and may survive for prolonged periods under conditions that would rapidly destroy the motile trophozoite. The majority of amebas belong to free-living genera. They are widely distributed in nature, being found in literally all bodies of standing fresh water. Few free-living amebas produce human disease, although two genera, *Naegleria* and *Acanthamoeba,* have been implicated occasionally as causes of meningoencephalitis and keratitis.

Several genera of amebas, including *Entamoeba, Endolimax,* and *Iodamoeba,* are obligate parasites of the human alimentary tract and are passed as cysts from host to host by the fecal–oral route. Several are devoid of mitochondria, presumably because of the anaerobic conditions under which they exist in the colon. Only one, *Entamoeba histolytica,* regularly produces disease; it has been recently subdivided into two morphologically identical but genetically distinct species, an invasive pathogen that retains the species appellation “histolytica” and a commensal organism, now designated *E. dispar*. The two species can be differentiated by isoenzyme analysis, antibodies to surface antigens, and DNA markers.

## ENTAMOEBA HISTOLYTICA

PARASITOLOGY

### MORPHOLOGY AND PHYSIOLOGY

*E. histolytica* possesses both trophozoite and cyst forms. The trophozoites are microaerophilic, dwell in the lumen or wall of the colon, feed on bacteria and tissue cells, and multiply rapidly in the anaerobic environment of the gut. When diarrhea occurs, the trophozoites are passed unchanged in the liquid stool. Here they can be recognized by their size (12 to 20 $\mu$m in diameter); directional motility; granular, vacuolated

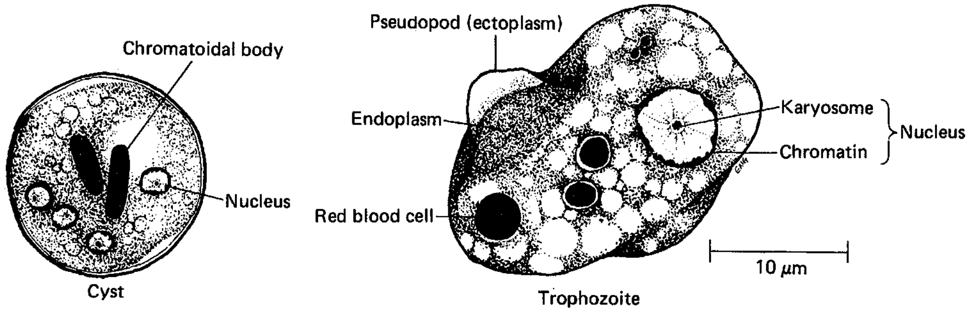


FIGURE 53–1
*Entamoeba histolytica.*

endoplasm; and sharply demarcated, clear ectoplasm with finger-like pseudopods. Invasive strains tend to be larger and may contain ingested erythrocytes within their cytoplasm (Fig 53–1). Appropriate stains reveal a 3- to 5-μm nucleus with a small central karyosome or nucleolus and fine regular granules evenly distributed around the nuclear membrane (peripheral chromatin). Electron microscopic studies demonstrate microfilaments, an external glycocalyx, and cytoplasmic projections thought to be important for attachment.

Trophozoites multiply rapidly in the gut

With normal stool transit time, trophozoites usually encyst before leaving the gut. Initially, a cyst contains a single nucleus, a glycogen vacuole, and one or more large, cigar-shaped ribosomal clusters known as chromatoid bodies. With maturation, the cyst becomes quadrinucleate, and the cytoplasmic inclusions are absorbed. In contrast to the fragile trophozoite, mature cysts can survive environmental temperatures up to 55°C, chlorine concentrations normally found in municipal water supplies, and normal levels of gastric acid. *E. histolytica* can be differentiated from the other amebas of the gut by its size, nuclear detail, and cytoplasmic inclusions (Table 53–1).

Cysts are hardy; can survive in chlorinated water supply

TABLE 53–1

## Some Differential Characteristics of *Entamoeba* Species

| CHARACTERISTICS | *E. HISTOLYTICA* | *E. HARTMANNI* | *E. COLI* |
|---|---|---|---|
| **Trophozoites** | | | |
| Cytoplasm | Differentiated[a] | Differentiated | Undifferentiated |
| Nucleus | | | |
| Peripheral chromatin | Fine | Fine | Coarse, irregular |
| Karyosome | Small, central | Small, central | Large, eccentric |
| Ingested particles | | | |
| Bacteria | No | — | Yes |
| Red blood cells | Yes | No | No |
| Size | >12 μm | <12 μm | >12 μm |
| **Cysts** | | | |
| Nuclei[b] | 1–4 | 1–4 | 1–8 |
| Chromatoid bodies | Rods | Rods | Splinters |
| Size | >10 μm | <10 μm | >10 μm |

[a] Sharp differentiation between ectoplasm and endoplasm.

[b] Fine structure similar to that of trophozoites.

## LIFE CYCLE

Humans are the principal hosts and reservoirs of *E. histolytica*. Transmission from person to person occurs when a parasite passed in the stool of one host is ingested by another. Because the trophozoites die rapidly in the external environment, successful passage is achieved only by the cyst. Human hosts may pass up to 45 million cysts daily. Although the average infective dose exceeds 1000 organisms, ingestion of a single cyst has been known to produce infection. After passage through the stomach, the cyst eventually reaches the distal small bowel. Here the cyst wall disintegrates, releasing the quadrinucleate parasite, which divides to form eight small trophozoites that are carried to the colon. Colonization is most intense in areas of fecal stasis such as the cecum and rectosigmoid but may be found throughout the large bowel.

Humans are the hosts and reservoir; fecal–oral transmission

## LABORATORY GROWTH

Trophozoites are facultative anaerobes that require complex media for growth. Most require the addition of live bacteria for successful isolation. Sterile culture techniques (axenic) have been developed, however, and are essential for the preparation of the purified antigens required for serologic testing, zymodeme typing, and characterization of virulence factors. Such techniques are generally available only in research laboratories.

Facultative anaerobes

# AMEBIASIS

CLINICAL CAPSULE

Amebiasis may be asymptomatic or produce intermittent diarrhea with abdominal pain. Invasion of the mucosa is typical and may spread to the liver, where an abscess is produced.

## EPIDEMIOLOGY

*E. histolytica* infection rates are higher in warm climates, particularly in areas where the level of sanitation is low. Worldwide, this organism is thought to produce more deaths than any other parasite, except those that cause malaria and schistosomiasis. Reports of amebic liver abscess, for instance, emanate primarily from Mexico, western South America, South Asia, and West and South Africa. For reasons apparently unrelated to exposure, symptomatic illness is much less common in women and children than in men.

Worldwide infection; highest rates in warmer climates

Although stool surveys in the United States indicate that 1 to 5% of the population harbors *Entamoeba,* the vast majority of these are now known to be colonized with the nonpathogenic *E. dispar*. The incidence of invasive amebiasis in the United States decreased sharply over several decades, reaching a nadir in 1974. Since then, the numbers have increased steadily. It is now seen particularly in institutionalized individuals, Indian reservations, migrant labor camps, victims of acquired immunodeficiency syndrome (AIDS), and travelers to endemic areas.

Invasive disease rare in United States

Symptomatic amebiasis is usually sporadic, the result of direct person-to-person fecal–oral spread under conditions of poor personal hygiene. Venereal transmission is seen in male homosexuals, presumably the result of oral–anal sexual contact. Food- and water-borne spread occur, occasionally in epidemic form. Such outbreaks, however, are seldom as explosive as those produced by pathogenic intestinal bacteria. One outbreak of intestinal amebiasis was due to colonic irrigation at a chiropractic clinic.

Fecal–oral spread linked to poor hygiene

Food and water are other modes of transmission

## PATHOGENESIS

A number of virulence factors have been identified in *E. histolytica*. In an experimental setting, invasiveness correlates well with endocytic capacity, the production of

Virulence determinants include lectin-mediated adherence to mucosa and capacity to lyse host cells

extracellular proteinases capable of activating complement and degrading collagen, the presence of a galactose-specific lectin apparently capable of mediating attachment of the organism to colonic mucosa, and perhaps most importantly, the capacity to lyse host cells on contact. The latter phenomenon is initiated by the galactose-specific lectin-mediated adherence of the trophozoite to a target cell. Following adherence, the ameba releases a pore-forming protein that polymerizes in the target cell membrane, forming large tubular lesions. Cytolysis rapidly follows.

Most infected individuals symptom free

Colonic microflora may influence invasiveness

Virulence increased with passage through humans

In most cases of infections, however, tissue damage is minimal, and the host remains symptom free, suggesting that host factors may modulate the invasiveness of virulent strains. These factors are still poorly understood, but changes in host resistance, the colonic milieu, or the parasite itself may amplify tissue damage and clinical manifestations. Protein malnutrition, high-carbohydrate diets, corticosteroid administration, childhood, and pregnancy all appear to render the host more susceptible to invasion. Certain colonic bacteria appear to enhance invasiveness, possibly by providing a more favorable redox potential for survival and multiplication or by facilitating the adherence of the parasite to colonic mucosa. Finally, it is known that the pathogenic strains in the tropics are more invasive than those isolated in temperate areas, possibly because poor sanitation results in more frequent passage through humans.

## PATHOLOGY

Mucosal ulceration with little inflammatory response

Flask-like ulcers extend to submucosa

Amebomas and metastatic amebic abscesses in a few cases

Amebas contact and lyse colonic epithelial cells, producing small mucosal ulcerations. There is little inflammatory response other than edema and hyperemia, and the mucosa between ulcers appears normal. Trophozoites are present in large numbers at the junction between necrotic and viable tissue. Once the lesion penetrates below the superficial epithelium, it meets the resistance of the colonic musculature and spreads laterally in the submucosa, producing a flask-like lesion with a narrow mucosal neck and a large submucosal body. It eventually compromises the blood supply of the overlying mucosa, resulting in sloughing and a large necrotic ulcer. Extensive ulceration leads to secondary bacterial infection, formation of granulation tissue, and fibrotic thickening of the colon. In approximately 1% of patients, the granulation tissue is organized into large, tumor-like masses known as amebomas. The major sites of involvement, in order of frequency, are the cecum, ascending colon, rectum, sigmoid, appendix, and terminal ileum. Amebas may also enter the portal circulation and be carried to the liver or, more rarely, to the lung, brain, or spleen. In these organs, liquefaction necrosis leads to the formation of abscess cavities.

## IMMUNITY

Although *E. histolytica* elicits both humoral and cellular immune responses in humans, it is still not clear which, and to what degree, these responses are capable of modulating initial infection or thwarting reinfection. In endemic areas, the prevalence of gastrointestinal colonization increases with age, suggesting that the host is incapable of clearing *E. histolytica* from the gut. However, the relative infrequency with which populations living in these areas suffer repeated bouts of severe amebic colitis or liver abscess indicates that those who experience such infections have protection against recurrent disease.

Immunity is incomplete and does not correlate with antibody response

Trophozoites shed antibody and resist complement lysis

Patients with invasive disease are known to produce high levels of circulating antibodies. Nevertheless, there is no correlation between the presence or concentration of such antibodies and protective immunity, possibly because pathogenic *E. histolytica* trophozoites have the capacity to aggregate and shed attached antibodies and are resistant to the lytic action of complement. The susceptibility to invasive amebiasis of malnourished populations, pregnant women, steroid-treated individuals, and AIDS patients indicates that cell-mediated immune mechanisms may be directly involved in the control of tissue invasion.

Pathogenic *E. histolytica* strains produce a lectin-like substance that is mitogenic for lymphocytes. It has been suggested that this substance could stimulate viral replication of human immunodeficiency virus–infected lymphocytes as does another mitogen, phytohemagglutinin.

# AMEBIASIS: CLINICAL ASPECTS

## MANIFESTATIONS

Individuals who harbor *E. histolytica* are usually clinically well. In most cases, particularly in the temperate zones, the organism is avirulent, living in the bowel as a normal commensal inhabitant. Spontaneous disappearance of amebas, over a period of weeks to months, among such patients is common and perhaps universal. Serologic data, however, suggest that some asymptomatic carriers possess virulent strains and incur minimal tissue invasion. In this population, the infection may eventually progress to produce overt disease.

Relationship usually commensal

Diarrhea, flatulence, and cramping abdominal pain are the most frequent complaints of symptomatic patients. The diarrhea is intermittent, alternating with episodes of normality or constipation over a period of months to years. Typically, the stool consists of one to four loose to watery, foul-smelling passages that contain mucus and blood. Physical findings are limited to abdominal tenderness localized to the hepatic, ascending colonic, and cecal areas. Sigmoidoscopy reveals the typical ulcerations with normal intertwining mucosa.

Diarrhea, flatulence, and abdominal pain most common

Ulcerations with mucus and blood in stool occur in fulminant disease

Fulminating amebic dysentery is less common. It may occur spontaneously in debilitated or pregnant individuals or be precipitated by corticosteroid therapy. Its onset is often abrupt, with high fever, severe abdominal cramps, and profuse diarrhea. Most commonly, abscesses occur singly and are localized to the upper outer quadrant of the right lobe of the liver. This localization results in the development of point tenderness overlying the cavity and elevation of the right diaphragm. Liver function is usually well preserved. Isotopic or ultrasound scanning confirms the presence of the lesion. Needle aspiration results in the withdrawal of reddish-brown, odorless fluid free of bacteria and polymorphonuclear leukocytes; trophozoites may be demonstrated in the terminal portion of the aspirate.

Hepatic abscess may have acute or insidious onset

Approximately 5% of all patients with symptomatic amebiasis present with a liver abscess. Ironically, fewer than one half can recall significant diarrheal illness. Although *E. histolytica* can be demonstrated in the stools of 72% of patients with amebic liver abscess when a combination of serial microscopic examinations and culture is used, routine microscopic examination of the stool detects less than half of these. Complications relate to the extension of the abscess into surrounding tissue, producing pneumonia, empyema, or peritonitis. Extension of an abscess from the left lobe of the liver to the pericardium is the single most dangerous complication. It may produce rapid cardiac compression (tamponade) and death or, more commonly, a chronic pericardial disease that may be confused with congestive cardiomyopathy or tuberculous pericarditis.

Hepatic abscess may extend to other tissues

## DIAGNOSIS

The microscopic diagnosis of intestinal amebiasis depends on the identification of the organism in stool or sigmoidoscopic aspirates. Because trophozoites appear predominantly in liquid stools or aspirates, a portion of such specimens should be fixed immediately to ensure preservation of these fragile organisms for stained preparations. The specimen may then be examined in wet mount for typical motility, concentrated to detect cysts, and stained for definitive identification. If trophozoites or cysts are seen, they must be carefully differentiated from those of the commensal parasites, particularly *E. hartmanni* and *E. coli* (see Table 53–1). *E. histolytica* trophozoites can be differentiated from those of *E. dispar* only by the presence of ingested erythrocytes in the former; the cysts appear identical.

Stools examined for trophozoites and cysts in stained or wet preparations

*E. histolytica* trophozoites ingest erythrocytes; *E. dispar* trophozoites do not

Recently, sensitive and specific stool antigen tests for *E. histolytica* have become commercially available; their value in the clinical diagnosis of amebiasis, when compared to microscopic examination, is now clear. Although cultural and polymerase chain reaction techniques are somewhat more sensitive, they are not widely available in most clinical laboratories.

Enzyme immunoassay and other methods can detect antigen in stool

The diagnosis of extraintestinal amebiasis is more difficult, because the parasite usually cannot be recovered from stool or tissue. Serologic tests are therefore of paramount importance. Typically, results are negative in asymptomatic patients, suggesting that tissue invasion is required for antibody production. Most patients with symptomatic intestinal disease and more than 90% with hepatic abscess have high levels of antiamebic antibodies. Unfortunately, these titers may persist for months to years after an acute infection, making the interpretation of a positive test difficult in endemic areas. At present, the indirect hemagglutination test and enzyme immunoassays using antigens derived from axenically grown organisms appear to be the most sensitive. Several rapid tests, including latex agglutination, agar diffusion, and counterimmunoelectrophoresis, are available to smaller laboratories.

Extraintestinal amebiasis usually demonstrates high antibody levels

## TREATMENT

Treatment is directed toward relief of symptoms, blood and fluid replacement, and eradication of the organism. The need to eliminate the parasite in asymptomatic carriers remains uncertain. The drug of choice for eradication is metronidazole. It is effective against all forms of amebiasis, but should be combined with a second agent, such as diloxanide, to improve cure rates in intestinal disease and diminish the chance of recrudescent disease in hepatic amebiasis. Specific contraindications to the use of metronidazole are given in Chapter 54 in the section on trichomoniasis.

Metronidazole combined with other agents

## PREVENTION

Because the disease is transmitted by the fecal–oral route, efforts should be directed toward sanitary disposal of human feces and improvement in personal hygienic practices. In the United States, this applies particularly to institutionalized patients and to camps for migrant farm workers. Male homosexuals should be made aware that certain sexual practices substantially increase their risk of amebiasis and other infections.

# *Naegleria* and *Acanthamoeba* Infections

## AMEBIC MENINGOENCEPHALITIS

Primary amebic meningoencephalitis is caused by free-living amebas belonging predominately to the *Naegleria* and *Acanthamoeba* genera. The disease produced by the former has been better defined; it affects children and young adults, appears to be acquired by swimming in fresh water, and is almost always fatal. *Acanthamoeba* meningoencephalitis is a subacute or chronic illness that also is usually fatal. *Naegleria* species are found in large numbers in shallow fresh water, particularly during warm weather. *Acanthamoeba* species are found in soil and in fresh and brackish water, and they have been recovered from the oropharynx of asymptomatic humans.

Meningoencephalitis due to free-living amebas

Warm weather and brackish water favor amebas

Approximately 140 cases of *Naegleria* meningoencephalitis have been reported, primarily in Great Britain, Belgium, Czechoslovakia, Australia, New Zealand, India, Nigeria, and the United States. Serologic studies suggest that inapparent infections are much more common. Most cases in the United States have occurred in the southeastern states. Characteristically, the patients have fallen ill during the summer after swimming or waterskiing in small, shallow, freshwater lakes. The Czechoslovakian cases followed swimming in a chlorinated indoor pool, and several have occurred after bathing in hot mineral water. A recent report from Africa suggests the disease may have been acquired by inhaling airborne cysts during the dry, windy season in the sub-Sahara.

*Naegleria* infections associated with freshwater swimming

Histologic evidence suggests that *Naegleria* traverses the nasal mucosa and the cribriform plate to the central nervous system. Here the organism produces a severe purulent, hemorrhagic inflammatory reaction that extends perivascularly from the

Passage to central nervous system across cribriform plate olfactory bulbs to other regions of the brain. The infection is characterized by the rapid onset of severe bifrontal headache, seizures, and at times, abnormalities in taste or smell. The disease runs an inexorably downhill course to coma, ending fatally within a few days.

A careful examination of the cerebrospinal fluid often provides a presumptive diagnosis of *Naegleria* infection. The fluid is usually bloody and demonstrates an intense neutrophilic response. The protein level is elevated and the glucose level decreased. No bacteria can be demonstrated on stain or culture. Early examination of a wet mount preparation of unspun spinal fluid reveals typical trophozoites. Staining with specific fluorescent antibody confirms the identification. The organism can usually be isolated on agar plates seeded with a Gram-negative bacillus (to feed the amebas) or grown axenically in tissue culture. To date, there are reports of only four patients who have survived a *Naegleria* infection. All were diagnosed early; and treated with high-dose amphotericin B along with rifampin.

Purulent bloody cerebrospinal fluid containing *Naegleria* trophozoites

The epidemiology of *Acanthamoeba* encephalitis has not been clearly defined. Infections usually involve older, immunocompromised persons, and a history of freshwater swimming is generally absent. The ameba probably reaches the brain by hematogenous dissemination from an unknown primary site, possibly the respiratory tract, skin, or eye. Metastatic lesions have been reported. Histologically, *Acanthamoeba* infections produce a diffuse, necrotizing, granulomatous encephalitis, with frequent involvement of the midbrain. Both cysts and trophozoites can be found in the lesions. Cutaneous ulcers and hard nodules containing amebas have been detected in AIDS patients.

Acanthamoeba affects older immunocompromised persons

Granulomatous encephalitis with cysts and trophozoites

The clinical course of *Acanthamoeba* disease is more prolonged than that of *Naegleria* infection and occasionally ends in spontaneous recovery; the disease in immunocompromised hosts is invariably fatal. The spinal fluid usually demonstrates a mononuclear response. Amebas can occasionally be visualized in or cultured from the cerebrospinal fluid or biopsy specimens. Fluorescein-labeled antiserum is available from the Centers for Disease Control and Prevention. Definitive diagnosis is usually made histologically after death. *Acanthamoeba* species are sensitive to a variety of agents, but studies of clinical efficacy have not been performed.

More prolonged disease with occasional spontaneous recovery

## OTHER *ACANTHAMOEBA* INFECTIONS

Skin lesions, uveitis, and corneal ulcerations have also been reported. The latter are serious, producing a chronic progressive ulcerative lesion that may result in blindness. Infection commonly follows mild corneal trauma; most recently reported cases have been in users of soft contact lenses. Clinically, severe ocular pain, a paracentral ring infiltrate of the cornea, and recurrent epithelial breakdown are helpful in distinguishing this entity from the more common herpes simplex keratitis. The diagnosis can be confirmed by demonstrating typical wrinkled, double-walled cysts in corneal biopsies or scrapings using wet mounts, stained smears, and/or fluorescent antibody techniques. Culture of corneal tissue and contact lenses is frequently successful when the laboratory is given time to prepare satisfactory media. Chemotherapy has generally been ineffective unless given very early in the course of infection. Although a combination of corneal transplantation and chemotherapy may be successful later in the course of the disease, enucleation of the eye may be necessary to cure advanced infections. The drugs of choice are propamidine and neomycin eyedrops administered alternately for a period of several months. Successful use of clotrimazole has been recently reported.

Corneal ulcerations associated with contact lens use

## ADDITIONAL READING

Chesley AJ, Craig CF, Fishbein M, et al. Amebiasis outbreak in Chicago. Report of a special committee. *JAMA* 1934;102:369–372. A description of the best-known outbreak of amebiasis in the United States. Fourteen hundred clinical infections and 100 deaths resulted from an inadvertent connection between the water supply and sewage in two Chicago hotels.

Duma RJ, Helwig WB, Martinez AJ. Meningoencephalitis and brain abscess due to a free-living amoeba. *Ann Intern Med* 1978;88:468–473. A useful case report and discussion regarding the taxonomic criteria used to identify free-living amebas producing human disease.

Haque R, et al. Comparison of PCR, isoenzyme analysis and antigen detection for diagnosis of *Entamoeba histolytica* infection. *J Clin Microbiol* 1998;36:449.

Haque R, et al. The global problem of amebiasis: current status, research needs and opportunities for progress. *Rev Infect Dis* 1986;8:218–272. This series of five papers covers the status, epidemiology, pathogenesis, immunology, and diagnosis of amebiasis. It is the most comprehensive review of *E. histolytica* infections.

Moore MB, McCulley JP, Luckenbach M. *Acanthamoeba* keratitis associated with soft contact lenses. *Am J Ophthalmol* 1985;100:396–403. This report of three patients who developed *Acanthamoeba* keratitis discusses the relationship between use of contact lenses and this disease, reviews the literature, and discusses diagnostic and therapeutic approaches.

Petri WA, Singh W. Diagnosis and management of amebiasis. *Clin Infect Dis* 1999; 29:1117.

Sison JP, et al. Disseminated *Acanthamoeba* infection in patients with AIDS: case reports and review. *Clin Infect Dis* 1995;20:1207.

Spice WM, Acker JP. The amoeba enigma. *Parasitol Today* 1992;8:402–406. This brief paper clearly and concisely reviews the evidence for and against the presence of distinct pathogenic and nonpathogenic forms of *E. histolytica*.

CHAPTER 54

# Flagellates

JAMES J. PLORDE

Like their amebic cousins, flagellate protozoa are widespread in nature, multiply by binary fission, and move about by means of cytoplasmic organelles of locomotion. Motility, however, is distinctly more vigorous among this group of organisms because of the efficiency of their locomotive apparatus, the flagellum. This organelle arises from an intracellular focus known as a blepharoplast, extends to the cell wall as a filamentous axoneme, and continues extracellularly as the free flagellum. In some species, the blepharoplast is paired with a second cytoplasmic structure known as a parabasal body. This structure is believed to be composed of modified mitochondria responsible for the control of flagellar movement. Both structures stain with nucleic acid stains, and they are known collectively as the kinetoplast.

In many flagellates the axoneme, before exiting from the cell, lifts a segment of external wall into a longitudinal fold. This undulating membrane is thrown into movement as the organism progresses, often imparting to it a characteristic rotary motion. The long, whip-like free flagella may be single or multiple. The number is distinctive for individual species. When more than one is present, each has its own associated blepharoplast and axoneme.

Although a number of flagellate genera parasitize humans, only four, *Trichomonas, Giardia, Leishmania,* and *Trypanosoma,* commonly induce disease. *Trichomonas* and *Giardia* are noninvasive organisms that inhabit the lumina of the genitourinary or gastrointestinal tract and are spread without benefit of an intermediate host. Disease is of low morbidity and cosmopolitan distribution. *Leishmania* and *Trypanosoma,* on the other hand, are invasive blood and tissue parasites that produce highly morbid, frequently lethal diseases. These hemoflagellates require an intermediate insect host for their transmission. As a result, their associated disease states are limited to the semitropical and tropical niches of these intermediate hosts.

## *NONINVASIVE LUMINAL FLAGELLATES*

Luminal flagellates can be found in the mouth, vagina, or intestine of almost all vertebrates, and it is common for an animal host to harbor more than one species. Humans may serve as host and reservoir to eight species (Table 54–1), but only two cause disease. Of these, *Giardia lamblia* inhabits the intestinal tract, and *Trichomonas vaginalis* inhabits the vagina and genital tract.

Found in flora of vertebrates

TABLE 54–1

**Luminal Flagellates Infecting Humans**

| Flagellate | Pathogenicity to Humans | Site |
|---|---|---|
| *Giardia lamblia* | + | Intestine |
| *Dientamoeba fragilis* | ? | Intestine |
| *Chilomastix mesnili* | − | Intestine |
| *Enteromonas hominis* | − | Intestine |
| *Retortamonas intestinalis* | − | Intestine |
| *Trichomonas hominis* | − | Intestine |
| *Trichomonas tenax* | − | Mouth |
| *Trichomonas vaginalis* | + | Vagina |

Morphology and rapid motility are distinctive

These organisms are elongated or oval in shape and typically measure 10 to 20 $\mu$m in length. They often possess a rudimentary cytostome (mouth aperture) and organelles such as sucking discs or axostyles, which help them maintain their intraluminal position. They are readily recognized in body fluid or excreta by their rapid motility, and some can be specifically identified in unstained preparations. All can be cultivated on artificial media.

May or may not have cyst stage

Some luminal flagellates, most notably *T. vaginalis,* possess only a trophozoite stage and are passed from host to host by direct physical contact. Most, including *G. lamblia,* possess both trophozoite and cyst forms. The latter, which is the infective form, is transmitted via the fecal–oral route. Human-to-human infection is thus found in populations where inadequate sanitation or poor personal hygiene favors spread.

# *Trichomonas vaginalis*

## PARASITOLOGY

Three *Trichomonas* species have similar morphology

Three members of the genus *Trichomonas* parasitize humans (see Table 54–1), but only *T. vaginalis* is an established pathogen. The three species closely resemble one another morphologically, but confusion in identification is rare because of the specificity of their habitats.

Protruding axostyle may mediate attachment

The *T. vaginalis* trophozoite (Fig 54–1) is oval and typically measures 7 by 15 $\mu$m. Organisms up to twice this size are occasionally recovered from asymptomatic patients and from cultures. In stained preparations, a single, elongated nucleus and a small cytostome are observed anteriorly. Five flagella arise nearby. Four immediately exit the cell. The fifth bends back and runs posteriorly along the outer edge of an abbreviated undulating membrane. Lying along the base of this membrane is a cross-striated structure known as the costa. A conspicuous microtubule containing a supporting rod or axostyle bisects the trophozoite longitudinally and protrudes through its posterior end. It is thought that the pointed tip of this structure is useful for attachment, and it may be responsible for the tissue damage produced by the parasite. In unstained wet mounts, *T. vaginalis* is identified by its axostyle and jerky, nondirectional movements.

Cultivable in vitro

Lacks cyst form but survives a few hours outside host

The organism can be grown on artificial media under anaerobic conditions at pH 5.5 to 6.0. Soluble nutrients are absorbed across the cell membrane. Particulate material, including bacteria, leukocytes, and occasional erythrocytes, may be ingested through any area of the cell surface. A variety of carbohydrates are fermented by pathways similar to those of anaerobic bacteria. Although it lacks a cyst form, the trophozoite can survive outside of the human host for 1 to 2 hours on moist surfaces. In urine, semen, and water,

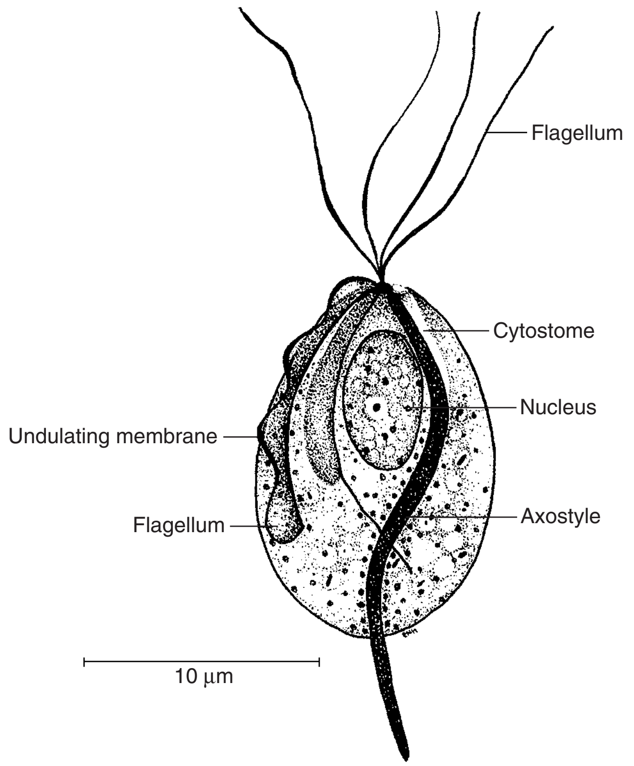


FIGURE 54–1
*Trichomonas vaginalis.*

it is viable for up to 24 hours, making it one of the most resistant of protozoan trophozoites. Attempts to infect laboratory animals have met with limited success.

## TRICHOMONIASIS

CLINICAL CAPSULE

Trichomoniasis is a sexually transmitted disease, which produces a vaginitis with pain, discharge, and dysuria. The infection fluctuates over weeks to months. Men are usually asymptomatic but may have urethritis or prostatitis.

### EPIDEMIOLOGY

Trichomoniasis is a cosmopolitan disease usually transmitted by sexual intercourse. It is estimated that 3 million women in the United States and 180 million worldwide acquire this disease annually, and 25% of sexually active women become infected at some time during their lives; 30 to 70% of their male sexual partners are also parasitized, at least transiently. As would be expected, the likelihood of acquiring the disease correlates directly with the number of sexual contacts. Infection is rare in adult virgins, whereas rates as high as 70% are seen among prostitutes, sexual partners of infected patients, and individuals with other venereal diseases. In women, the peak incidence is between 16 and 35 years of age, but there is a relatively high prevalence in the 30- to 50-year age group.

Transmission usually sexual

Prevalence linked to sexual activity

Nonvenereal transmission is uncommon. Transfer of organisms on shared washcloths may explain, in part, the high frequency of infection seen among institutionalized women. Female neonates are occasionally noted to harbor *T. vaginalis,* presumably acquiring it during passage through the birth canal. High levels of maternal estrogen produce a transient decrease in the vaginal pH of the child, rendering it more susceptible to

Nonvenereal transmission uncommon

colonization. Within a few weeks, estrogen levels drop, the vagina assumes its premenarcheal state, and the parasite is eliminated.

## PATHOGENESIS AND IMMUNITY

Parasite damages epithelial cells on contact

Direct contact of *T. vaginalis* with the squamous epithelium of the genitourinary tract results in destruction of the involved epithelial cells and the development of a neutrophilic inflammatory reaction and petechial hemorrhages. The precise pathogenesis of these changes is unknown. The organism is not invasive, and extracellular toxins have never been demonstrated. The expression of a 200-kd parasitic glycoprotein, however, has been found to correlate with clinical manifestations. Changes in the microbial, hormonal, and pH environment of the vagina as well as factors inherent to the infecting parasite are thought to modulate the severity of the pathologic changes. Although humoral, secretory, and cellular immune reactions can be demonstrated in most infected women, they are of little diagnostic help and do not appear to produce clinically significant immunity.

# TRICHOMONIASIS: CLINICAL ASPECTS

## MANIFESTATIONS

Chronic vaginitis lasting weeks to months

In women, *T. vaginalis* produces a persistent vaginitis. Although up to 50% are asymptomatic at the time of diagnosis, most develop clinical manifestations within 6 months. Approximately 75% develop a discharge, which is typically accompanied by vulvar itching or burning (50%), dyspareunia (50%), dysuria (50%), and a disagreeable odor (10%). Although fluctuating in intensity, symptoms usually persist for weeks or months. Commonly, manifestations worsen during menses and pregnancy. Eventually, the discharge subsides, even though the patient may continue to harbor the parasite. In symptomatic patients, physical examination reveals reddened vaginal and endocervical mucosa. In severe cases, petechial hemorrhages and extensive erosions are present. A red, granular, friable endocervix (strawberry cervix) is a characteristic but uncommon finding. An abundant discharge is generally seen pooled in the posterior vaginal fornix. Although classically described as thin, yellow, and frothy in character, the discharge more frequently lacks these characteristics. Recent studies have demonstrated that trichomoniasis both increases the risk of preterm birth and enhances susceptibility to human immunodeficiency virus (HIV) infections.

Urethral and prostatic infection in men usually asymptomatic

The urethra and prostate are the usual sites of infection in men; the seminal vesicles and epididymis may be involved on occasion. Infections are usually asymptomatic, possibly because of the efficiency with which the organisms are removed from the urogenital tract by voided urine. Symptomatic men complain of recurrent dysuria and scant, nonpurulent discharge. Acute purulent urethritis has been reported rarely. Trichomoniasis should be suspected in men presenting with nongonococcal urethritis, or a history of either prior trichomonal infection or recent exposure to trichomoniasis.

## DIAGNOSIS

Wet mount examination for motile trophozoites sufficient in most symptomatic cases

The diagnosis of trichomoniasis rests on the detection and morphologic identification of the organism in the genital tract. Identification is accomplished most easily by examining a wet mount preparation for the presence of motile organisms. In women, a drop of vaginal discharge is the most appropriate specimen; in men, urethral exudate or urine sediment after prostate massage may be used. Although highly specific when positive, wet mounts have a sensitivity of only 50 to 60%. They are most likely to be negative in asymptomatic or mildly symptomatic patients and in women who have douched in the previous 24 hours. Giemsa- and Papanicolaou-stained smears provide little additional help. The recent introduction of a commercial system that allows direct, rapid microscopic examination without the need for daily sampling may ameliorate this situation. Direct immunofluorescent antibody staining has a sensitivity of 70 to 90%. Parasitic culture, while more sensitive, requires several days to complete and is frequently unavailable.

## TREATMENT

Oral metronidazole is extremely effective in recommended dosage, curing more than 95% of all infections. It may be given as a single dose or over 7 days. Simultaneous treatment of sexual partners may minimize recurrent infections, particularly when single-dose therapy is used for the index case. Because of the disulfiram-like activity of metronidazole, alcohol consumption should be suspended during treatment. The drug should never be used during the first trimester of pregnancy because of its potential teratogenic activity. Use in the last two trimesters is unlikely to be hazardous but should be reserved for patients whose symptoms cannot be adequately controlled with local therapies. High-dose, long-term metronidazole treatment has been shown to be carcinogenic in rodents. No association with human malignancy has been described to date, and in the absence of a suitable alternative drug, metronidazole continues to be used.

Metronidazole cures 95% of cases

# *Giardia lamblia*

## PARASITOLOGY

*G. lamblia* was first described by Anton von Leeuwenhoek 300 years ago when he examined his own diarrheal stool with one of the first primitive microscopes. It was not until the past several decades, however, that this cosmopolitan flagellate became widely regarded in the United States as a pathogen. Of the six other flagellated protozoans known to parasitize the alimentary tract of humans, only one, *Dientamoeba fragilis,* has been credibly associated with disease. Definitive confirmation or refutation of its pathogenicity will, it is hoped, not require the passage of another three centuries.

Unlike *T. vaginalis, Giardia* possesses both a trophozoite and a cyst form (Fig 54–2). It is a sting-ray–shaped trophozoite 9 to 21 $\mu$m in length, 5 to 15 $\mu$m in width, and 2 to 4 $\mu$m in thickness. When viewed from the top, the organism's two nuclei and central parabasal bodies give it the appearance of a face with two bespectacled eyes and a crooked mouth. Four pairs of flagella—anterior, lateral, ventral, and posterior—reinforce this

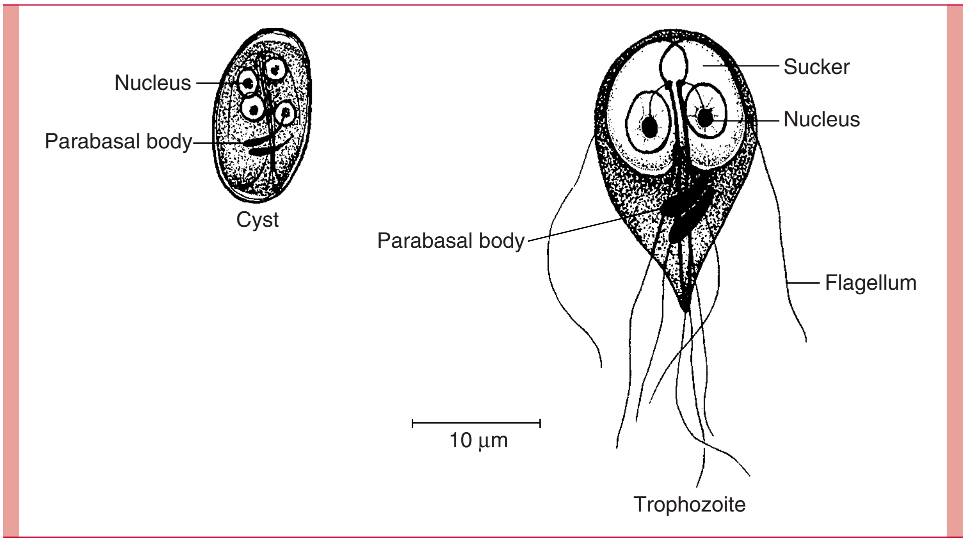


FIGURE 54–2
*Giardia lamblia.*

Trophozoite and cyst stages

Move about duodenum and jejunum with tumbling motility

image by suggesting the presence of hair and chin whiskers. These distinctive parasites reside in the duodenum and jejunum, where they thrive in the alkaline environment and absorb nutrients from the intestinal tract. They move about the unstirred mucous layer at the base of the microvilli with a peculiar tumbling or "falling leaf" motility or, with the aid of a large ventral sucker, attach themselves to the brush border of the intestinal epithelium. Unattached organisms may be carried by the fecal stream to the large intestine.

Cystic forms develop in colon

Resistant cysts transmitted from host to host

In the descending colon, if transit time allows, the flagella are retracted into cytoplasmic sheaths and a smooth, clear cyst wall is secreted. These forms are oval and somewhat smaller than the trophozoites. With maturation, the internal structures divide, producing a quadrinucleate organism harboring two sucking discs, four parabasal bodies, and eight axonemes (see Fig 54–2). When fixed and stained, the cytoplasm pulls away from the cyst wall in a characteristic fashion. The mature cysts, which are the infective form of the parasite, may survive in cold water for more than 2 months and are resistant to concentrations of chlorine generally used in municipal water systems. They are transmitted from host to host by the fecal–oral route. In the duodenum of a new host, the cytoplasm divides to produce two binucleate trophozoites.

Wide distribution in animal kingdom

Organisms of the genus *Giardia* are among the most widely distributed of intestinal protozoa; they are found in fish, amphibians, reptiles, birds, and mammals. At first, it was assumed that *Giardia* strains found in different animals were host specific; on this basis, some 40 different species were described. As it is now recognized that some strains can infect multiple animal hosts, the practice of assigning species status by the host from which the parasite was recovered is considered invalid. Unfortunately, there is still no general agreement on an alternate method of speciation. Three morphologically distinct groups of *Giardia* have been described on the basis of their central parabasal body morphology.

# GIARDIASIS

**Clinical Capsule**

Giardiasis, an intestinal infection acquired from untreated water sources, is most often symptomatic. When disease occurs, it is in the form of a diarrhea lasting up to 4 weeks with foul-smelling, greasy stools. Abdominal pain, nausea, and vomiting are also present.

## EPIDEMIOLOGY

Transmission facilitated by poor hygiene and IgA deficiency

High attack rates in day-care centers

Giardiasis frequent among male homosexuals

Giardiasis has a cosmopolitan distribution; its prevalence is highest in areas with poor sanitation and among populations unable to maintain adequate personal hygiene. In developing countries, infection rates may reach 25 to 30%; in the United States, *G. lamblia* is found in 4% of stools submitted for parasitologic examination, making it this country's most frequently identified intestinal parasite. All ages and economic groups are represented, but young children and young adults are preferentially involved. Children with immunoglobulin deficiencies are more likely to acquire the flagellate, possibly because of a deficiency in intestinal immunoglobulin A. Giardiasis is also common among attendees of day-care centers. Attack rates of over 90% have been seen in the ambulatory non–toilet-trained population (age, 1 to 2 years) of these institutions, suggesting direct person-to-person transmission of the parasite. The frequency with which secondary cases are seen among family contacts reinforces this probability. Undoubtedly, direct fecal spread is also responsible for the high infection rate among male homosexuals. In several recent studies, the prevalence of giardiasis and/or amebiasis in that population has ranged from 11 to 40% and is correlated closely with the number of oral–anal sexual contacts.

Water-borne and, less frequently, food-borne transmission of *G. lamblia* has also been documented, and probably accounts for the frequency with which American travelers to third world nations acquire infection. Unlike the typical bacterial diarrhea syndrome seen in travelers, the diarrhea begins late in the course of travel and may persist for several weeks. More than 20 water-borne outbreaks of giardiasis have also been reported in the United States. The sources have included untreated pond or stream water, sewage-contaminated municipal water supplies, and chlorinated but inadequately filtered water. In a few of these outbreaks, epidemiologic data have suggested that wild mammals, particularly beavers, served as the reservoir hosts. Domestic cats and dogs, which have recently been shown to have a high prevalence of *G. lamblia,* may also act as reservoirs for human infections.

Water- or food-borne traveler's diarrhea lasts for weeks

Beavers and other mammals possible sources

## PATHOGENESIS

Disease manifestations appear related to intestinal malabsorption, particularly of fat and carbohydrates. Disaccharidase deficiency with lactose intolerance, altered levels of intestinal peptidases, and decreased vitamin $B_{12}$ absorption have been demonstrated. The precise pathogenetic mechanisms responsible for these changes remain poorly understood. Mechanical blockade of the intestinal mucosa by large numbers of *Giardia,* damage to the brush border of the microvilli by the parasite's sucking disc, organism-induced deconjugation of bile salts, altered intestinal motility, accelerated turnover of mucosal epithelium, and mucosal invasion have all been suggested. None of these correlates well with clinical manifestations. Patients with severe malabsorption have jejunal colonization with enteric bacteria or yeasts, suggesting that these organisms may act synergistically with *Giardia.* Eradication of the associated microorganism, however, has not uniformly resulted in clinical improvement. Jejunal biopsies sometimes reveal a flattening of the microvilli and an inflammatory infiltrate, the severity of which correlates roughly with that of the clinical disease. Generally, both malabsorption and the jejunal lesions have been reversed with specific treatment. The demonstration of occasional trophozoites in the submucosa raises the possibility that these changes reflect T lymphocyte–mediated damage.

Basis for malabsorption and jejunal pathology remains uncertain

## IMMUNITY

Susceptibility to giardiasis has been related to several factors, including strain virulence, inoculum size, achlorhydria or hypochlorhydria, and immunologic abnormalities. In one experimental study, humans were challenged with varying doses from as few as 10 cysts. They were uniformly parasitized when 100 or more were ingested. Several workers have noted the frequency with which giardiasis occurs in achlorhydric and hypochlorhydric individuals. Although reinfection is common, the frequent occurrence of giardiasis in patients with immunologic diseases, plus the rarity with which it is seen in older adults, suggests that protective immunity, albeit incomplete, does develop in humans. Animal studies have demonstrated that *Giardia*-specific, secretory IgA (sIgA) antibodies inhibit attachment of trophozoites to intestinal epithelium, perhaps by blocking parasite surface lectins. Moreover, antitrophozoite IgM or IgG antibodies, plus complement, are known to be capable of killing *Giardia* trophozoites.

Predisposing factors include hypochlorhydria and immunocompromise

# GIARDIASIS: CLINICAL ASPECTS

## MANIFESTATIONS

In endemic situations, over two thirds of infected patients are asymptomatic. In acute outbreaks, this ratio of asymptomatic to symptomatic patients is usually reversed. When they do occur, symptoms begin 1 to 3 weeks after exposure; they typically include diarrhea, which is sudden in onset and explosive in character. The stool is foul smelling, greasy in appearance, and floats on water. It is devoid of blood or mucus. Upper abdominal cramping is common. Large quantities of intestinal gas produce abdominal distention, sulfuric eructations, and abundant flatus. Nausea, vomiting, and low-grade fever may be present. The acute illness generally resolves in 1 to 4 weeks; in children, however, it may persist for months, leading to significant malabsorption, weight loss, and malnutrition.

Subclinical infections common in endemic areas

Diarrhea, cramping, flatus, and greasy stools

Subacute and chronic infections with weight loss in adults

Lactose intolerance may persist

In many adults, the acute phase is often followed by a subacute or chronic phase characterized by intermittent bouts of mushy stools, flatulence, and "heartburn" and weight loss that persist for weeks or months. At times, patients presenting in this fashion deny having experienced the acute syndrome described previously. In the majority, symptoms and organisms eventually disappear spontaneously. It is not uncommon for lactose intolerance to persist after eradication of the organisms. This condition may be confused with an ongoing infection, and the patient may be subjected to unnecessary treatment.

## DIAGNOSIS

Demonstration of trophozoites and cysts in stool or duodenal aspirates diagnostic

EIAs detect *Giardia* antigen in stool

The diagnosis is made by finding the cyst in formed stool or the trophozoite in diarrheal stools, duodenal secretions, or jejunal biopsy specimens. In acutely symptomatic patients, the parasite can usually be demonstrated by examining one to three stool specimens, providing appropriate concentration and staining procedures are used. In chronic cases, excretion of the organism is often intermittent, making parasitologic confirmation more difficult. Many of these patients can be diagnosed by examining specimens taken at weekly intervals over 4 to 5 weeks. Alternatively, duodenal secretions can be collected and examined for trophozoites in trichrome or Giemsa-stained preparations. There are now a number of reliable, commercially available, enzyme immunoassays (EIAs) for the direct detection of parasite antigen in stool. They appear to be as sensitive and specific as microscopic examinations. The organism can be grown in culture, but the methods are not currently adaptable to routine diagnostic work.

## TREATMENT

Several drugs available

Close contacts should be examined

Four drugs are currently available for the treatment of giardiasis in the United States: quinacrine hydrochloride, metronidazole, furazolidone, and paromomycin. Quinacrine and metronidazole are somewhat more effective (70 to 95%) and are preferred for patients capable of ingesting tablets. Furazolidone is used by pediatricians because of its availability as a liquid suspension, but it has the lowest cure rate. These three agents require 5 to 7 days of therapy. Tinidazole, an oral agent not yet available in the United States, is safe and effective in single-dose treatment. Because of the potential of giardiasis for person-to-person spread, it is important to examine and, if necessary, treat close physical contacts of the infected patient, including playmates at nursery school, household members, and sexual contacts. None of the aforementioned agents should be used in pregnant women because of their potential teratogenicity. Paromomycin, a nonabsorbed but somewhat less effective agent, may be used in this circumstance.

## PREVENTION

Avoid drinking untreated surface water

Hikers should avoid ingestion of untreated surface water, even in remote areas, because of the possibility of contamination by feces of infected animals. Adequate disinfection can be accomplished with halogen tablets yielding concentrations higher than that generally achieved in municipal water systems. The safety of the latter results from additional flocculation and filtration procedures.

# BLOOD AND TISSUE FLAGELLATES

Two of the many genera of hemoflagellates are pathogenic to humans, *Leishmania* and *Trypanosoma.* They reside and reproduce within the gut of specific insect hosts. When these vectors feed on a susceptible mammal, the parasite penetrates the feeding site, invades the blood and/or tissue of the new host, and multiplies to produce disease. The life cycle is completed when a second insect ingests the infected mammalian blood or

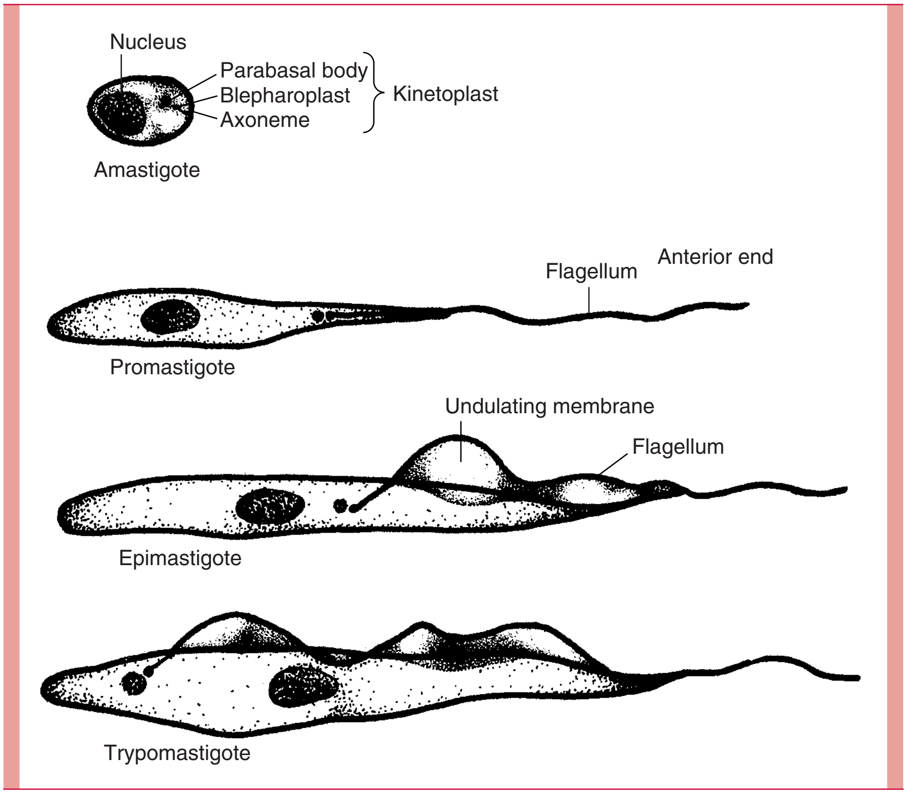


FIGURE 54–3
Stages in the life cycle of the hemoflagellates (Trypanosomidae).

tissue fluid. During the course of their passage through insect and vertebrate hosts, flagellates undergo developmental change. Within the gut of the insect (and in culture media), the organism assumes the promastigote (*Leishmania*) or epimastigote (*Trypanosoma*) form (Fig 54–3). These protozoa are motile, fusiform, and have a blunt posterior end and a pointed anterior from which a single flagellum projects. They measure 15 to 30 $\mu$m in length and 1.5 to 4.0 $\mu$m in width. In the promastigote, the kinetoplast is located in the anterior extremity and the flagellum exits from the cell immediately. The kinetoplast of the epimastigote, in contrast, is located centrally, just in front of the vesicular nucleus. The flagellum runs anteriorly in the free edge of an undulating membrane before passing out of the cell. In the mammalian host, hemoflagellates appear as trypomastigotes (*Trypanosoma*) or amastigotes (*Leishmania, T. cruzi*). The former circulate in the bloodstream and closely resemble the epimastigote form, except that the kinetoplast is in the posterior end of the parasite. The amastigote stage is found intracellularly. It is round or oval, measures 1.5 to 5.0 $\mu$m in diameter, and contains a clear nucleus with a central karyosome. Although it has a kinetoplast and an axoneme, there is no free flagellum.

Life cycle includes insect host stage

Promastigote and epimastigote forms in insects

Trypomastigote and amastigote forms in humans

The flagellated forms move in a spiral fashion, and all reproduce by longitudinal binary fission. The flagellum itself does not divide; rather, a second one is generated by one of the two daughter cells. The organisms use carbohydrate obtained from the body fluids of the host in aerobic respiration.

## *Leishmania*

### PARASITOLOGY

*Leishmania* species are obligate intracellular parasites of mammals. Several strains can infect humans; they are all morphologically similar, resulting in some confusion over their proper speciation. Definitive identification of these strains requires isoenzyme analysis, monoclonal antibodies, kinetoplast DNA buoyant densities, DNA hybridization, and DNA restriction endonuclease fragment analysis or chromosomal karyotyping using pulse-field electrophoresis. The many strains can be more simply placed in four major groups based on their serologic, biochemical, cultural, nosologic, and behavioral characteristics. For the sake of clarity, these groups will be discussed as individual species. Each, however, contains a variety of strains that have been accorded separate species or subspecies status by some authorities. The organisms can be propagated in hamsters and in a variety of commercially available liquid media.

Species morphologically similar; differ in molecular features

## DISEASE TRANSMISSION

Cutaneous ulcer or visceral infection (kala azar) the primary diseases

It is estimated that over 20 million people worldwide suffer from leishmaniasis and 1 to 2 million additional individuals acquire the infection annually. *Leishmania tropica* in the Old World and *L. mexicana* in the New World produce a localized cutaneous lesion or ulcer, known popularly as oriental sore and chiclero ulcer; *L. braziliensis* is the cause of American mucocutaneous leishmaniasis (espundia); and *L. donovani* is the etiologic agent of kala azar, a disseminated visceral disease.

All four groups transmitted by nocturnally feeding sandflies

Complement activation mediates attachment to macrophages

Intracellular survival by inhibiting macrophage killing mechanisms

Amastigotes released from macrophages can infect feeding sandfly

All four are transmitted by phlebotomine sandflies. These small, delicate, short-lived insects are found in animal burrows and crevices throughout the tropics and subtropics. At night, they feed on a wide range of mammalian hosts. Amastigotes ingested in the course of a meal assume the flagellated promastigote form, multiply within the gut, and eventually migrate to the buccal cavity. When the fly next feeds on a human or animal host, the buccal promastigotes are injected into the skin of the new host together with salivary peptides capable of inactivating host macrophages. Here, they activate complement by the classic (*L. donovani*) or alternative pathway and are opsonized with C3, which mediates attachment to the CR1 and CR3 complement receptors of macrophages. Following phagocytosis, the promastigotes lose their flagella and multiply as the rounded amastigote form within the phagolysosome. In stained smears, the parasites take on a distinctive appearance and have been termed Leishman–Donovan bodies. Intracellular survival is mediated by a surface lipophosphoglycan and an abundance of membrane-bound acid phosphatase, which inhibit the macrophage's oxidative burst and/or inactivate lysosomal enzymes. Continued multiplication leads to the rupture of the phagocyte and release of the daughter cells. Some may be taken up by a feeding sandfly; most invade neighboring mononuclear cells (Fig 54–4).

In localized cutaneous disease, cellular immune responses produce spontaneous cure

Mucocutaneous metastases in *L. braziliensis* infections

Continuation of this cycle results in extensive histiocytic proliferation. The course of the disease at this point is determined by the species of parasite and the response of the host's T cells. CD4+ T cells of the $T_H1$ type secrete interferon-$\gamma$ in response to leishmanial antigens. This, in turn, activates macrophages to kill intracellular amastigotes by the production of toxic nitric oxide. In the localized cutaneous forms of leishmaniasis, this immune response results in the development of a positive delayed skin (leishmanin) reaction, lymphocytic infiltration, reduction in the number of parasites, and, eventually,

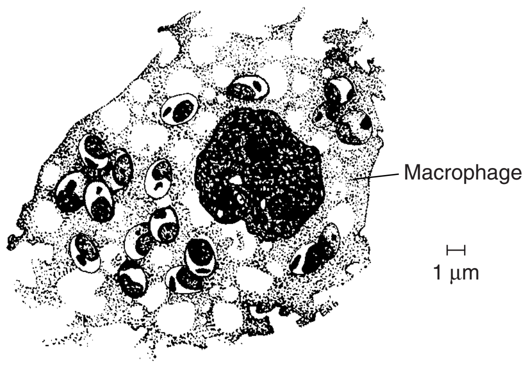


FIGURE 54–4
*Leishmania* within a mononuclear cell.

TABLE 54–2

**Immune Response to Leishmaniasis**

| Human Disease | Parasite | Leishmanin Skin Test | Number of Lymphocytes | Number of Parasites | Prognosis | Humoral Antibody Titer |
|---|---|---|---|---|---|---|
| Localized skin ulcer (oriental sore, chiclero ulcer, uta) | *L. tropica*<br>*L. mexicana* | Positive | Many | Few | Good | Low |
| Mucocutaneous lesions (espundia) | *L. braziliensis* | Positive | Many | Few | Poor | Low |
| Disseminated cutaneous | | | | | | |
| Ethiopian | *L. tropica*[a] | Negative | Few | Many | Poor | High |
| American | *L. mexicana*[a] | | | | | |
| Disseminated visceral (kala azar) | *L. donovani* | Negative | Few | Many | Poor | High |

[a] Different subspecies from those causing localized skin ulcers.

spontaneous disappearance of the primary skin lesion. In infections with *L. braziliensis,* this sequence may be followed weeks to months later by mucocutaneous metastases. These secondary lesions are highly destructive, presumably as a result of the host's hypersensitivity to parasitic antigens.

Some strains of *L. tropica* and *L. mexicana* fail to elicit an effective intracellular immune response in certain hosts. Such patients appear to have a selective suppressor T lymphocyte–mediated anergy to leishmanial antigens. Consequently, there is no infiltration of lymphocytes or decrease in the number of parasites. The skin test remains negative, and the skin lesions disseminate and become chronic (diffuse cutaneous leishmaniasis). In infections with *L. donovani,* there is a more dramatic inhibition of the $T_H1$ response. The leishmanial organisms are able to disseminate through the bloodstream to the visceral organs, possibly because of a relative resistance of *L. donovani* to the natural microbicidal properties of normal serum, and/or their ability to better survive at 37°C than strains of *Leishmania,* causing cutaneous lesions. Although dissemination is associated with the development of circulating antibodies, they do not appear to serve a protective function and may, via the production of immune complexes, be responsible for the development of glomerulonephritis. A simplified outline of the immune responses in different forms of leishmaniasis is presented in Table 54–2.

Lack of cellular immune response in disseminated and chronic infections

## LOCALIZED CUTANEOUS LEISHMANIASIS

### EPIDEMIOLOGY

The disease is a zoonotic infection of tropical and subtropical rodents. It is particularly common in areas of Central Asia, the Indian subcontinent, Middle East, Africa, the Mediterranean littoral, and Central and South America. In the latter area, *L. mexicana* infects several species of arboreal rodents. Humans become involved when they enter forested areas to harvest chicle for chewing gum and are bitten by infected sandflies. In the eastern hemisphere, the desert gerbil and other burrowing rodents serve as the reservoir hosts of *L. tropica.* Human infection occurs when rural inhabitants come in close contact with the burrows of these animals. In the Mediterranean area, southern Russia, and India, human disease involves urban dwellers, primarily children. In this setting, the domestic dog serves as the reservoir, although sandflies may also transmit *L. tropica* directly from human to human.

Geographic distribution related to human and rodent reservoirs

Canine reservoir in urban disease

## LOCALIZED CUTANEOUS LEISHMANIASIS

### MANIFESTATIONS

Chronic, self-limiting skin ulceration

Strain-specific immunity

Lesions usually appear on the extremities or face (the ear in cases of chiclero ulcer) weeks to months after the bite of the sandfly. They first appear as pruritic papules, often accompanied by regional lymphadenopathy. In a few months the papules ulcerate, producing painless craters with raised erythematous edges, sharp walls, and a granulating base. Satellite lesions may form around the edge of the primary sore and fuse with it. Multiple primary lesions are seen in some patients. Spontaneous healing occurs in 3 to 12 months, leaving a flat, depigmented scar. Occasionally the lesions fail to heal, particularly on the ears, leading to progressive destruction of the pinna. A permanent strain-specific immunity usually follows healing. Multiple, disseminated nonhealing lesions may be seen in patients with acquired immunodeficiency syndrome (AIDS).

### TREATMENT

Demonstration of Leishman–Donovan bodies or culture from tissue biopsy

In endemic areas, the diagnosis is made on clinical grounds and confirmed by the demonstration of the organism in the advancing edge of the ulcer. Material collected by biopsy, curettage, or aspiration is smeared and/or sectioned, stained, and examined microscopically for the pathognomonic Leishman–Donovan bodies. Material should also be cultured in liquid media. The leishmanin skin test becomes positive early in the course of the disease and remains so for life. Recently, it has been demonstrated that small numbers of *Leishmania* may be detected in tissue by the polymerase chain reaction (PCR), and strains distinguished with probes to kinetoplast DNA. These techniques, although not widely available, permit direct, rapid, and specific diagnosis of all leishmanial infections.

Patients with small, cosmetically minor lesions that do not involve the mucous membrane may be carefully followed without treatment. Pentavalent antimonial agents and liposomal amphotericin B have proved to be effective chemotherapeutic agents for individuals with more consequential lesions. Recently, ketoconazole and itraconazole, alone or in combination with the previously mentioned agents, have been found to be effective in some forms of cutaneous leishmaniasis. Bacterial superinfections are treated with appropriate antibiotics. Prophylactic measures include the control of the sandfly vector by use of insect repellents and fine mesh screening on dwellings.

## MUCOCUTANEOUS LEISHMANIASIS

### EPIDEMIOLOGY

Rodent reservoir of *L. braziliensis*

*L. braziliensis* causes a natural infection in the large forest rodents of tropical Latin America. Sandflies transmit the infection to humans engaged in military activities, road builders opening jungle areas for new settlements, and others.

## MUCOCUTANEOUS LEISHMANIASIS

### MANIFESTATIONS

Primary lesion metastasizes to oral and nasal areas

A primary skin lesion similar to oriental sore develops 1 to 4 weeks after sandfly exposure. Occasionally it undergoes spontaneous healing. More commonly, it progressively enlarges, often producing large vegetating lesions. After a period of weeks to years, painful, destructive, metastatic mucosal lesions of the mouth, nose, and occasionally the perineum, appear in 2 to 50% of patients. Sometimes, decades pass and the primary lesion totally resolves before the metastases manifest themselves. Destruction of the nasal septum produces the characteristic tapir nose. Erosion of the hard palate and larynx may render the patient aphonic. In blacks, the lesions are often large, hypertrophic, polypoid masses that deform the lips and cheeks. Fever, anemia, weight loss, and secondary bacterial infections are common. Mucosal lesions caused by other *Leishmania* species may be seen following visceral dissemination in AIDS patients.

## TREATMENT

The diagnosis is made by finding the organisms in the lesions as described for localized cutaneous leishmaniasis. Because the propensity to metastasize to mucocutaneous sites is specific to certain species and subspecies, precise identification of the responsible organism as described in the introduction is of clinical importance. The leishmanin skin test yields positive results, and most patients have detectable antibodies. As described for cutaneous leishmaniasis, it is now possible to provide a rapid, direct, species-specific diagnosis through the use of the PCR and probes to kinetoplast DNA.

Treatment is accomplished with the agents described later in the chapter for kala azar. Advanced lesions are often refractory, and relapse is common. Cured patients are immune to reinfection. Control measures, other than insect repellents and screening of dwellings, are impractical because of the sylvatic nature of the disease.

Detection of organisms as with cutaneous leishmaniasis

# DISSEMINATED VISCERAL LEISHMANIASIS (KALA AZAR)

## EPIDEMIOLOGY

Kala azar, which is caused by *L. donovani,* occurs in the tropical and subtropical areas of every continent except Australia. Its epidemiologic and clinical patterns vary from area to area. In Africa, rodents serve as the primary reservoir. Human cases occur sporadically, and the disease is often acute and highly lethal. In Eurasia and Latin America, the domestic dog is the most common reservoir. Human disease is endemic, primarily involves children, and runs a subacute to chronic course. In India, the human is the only known reservoir, and transmission is carried out by anthropophilic species of sandflies. The disease recurs in epidemic form at 20-year intervals, when a new cadre of nonimmune children and young adults appears in the community. There appears to be a high incidence of visceral leishmaniasis in patients with HIV infection. Presumably, HIV-induced immunosuppression either facilitates acquisition of the disease and/or allows reactivation of latent infection.

Marked geographic differences in reservoirs and disease severity

## PATHOGENESIS

After the host is bitten by an infected sandfly, the parasites disseminate in the bloodstream and are taken up by the macrophages of the spleen, liver, bone marrow, lymph nodes, skin, and small intestine. Histiocytic proliferation in these organs produces enlargement with atrophy or replacement of the normal tissue.

Parasites invade macrophages of reticuloendothelial system

# DISSEMINATED VISCERAL LEISHMANIASIS (KALA AZAR)

## MANIFESTATIONS

The majority of infections are asymptomatic; these become symptomatic years later during periods of host immunocompromise. Symptomatic disease most commonly manifests itself 3 to 12 months after acquisition of the parasite. It is often mild and self-limited. A minority of infected individuals develop the classic manifestations of kala azar. Fever, which is usually present, may be abrupt or gradual in onset. It persists for 2 to 8 weeks and then disappears, only to reappear at irregular intervals during the course of the disease. A double-quotidian pattern (two fever spikes in a single day) is a characteristic but uncommon finding. Diarrhea and malabsorption are frequent in Indian cases, resulting in progressive weight loss and weakness. Physical findings include enlarged lymph nodes and liver, massively enlarged spleen, and edema. In light-skinned individuals, a grayish pigmentation of the face and hands is commonly seen, which gives the disease its name (kala azar, black disease). Anemia with resulting pallor and tachycardia are typical in advanced cases. Thrombocytopenia induces petechial formation and mucosal bleeding. The peripheral leukocyte count is usually less than 4000/mm$^3$; agranulocytosis with secondary bacterial infections contributes to lethality. Serum immunoglobulin G levels are enormously elevated but play no protective role. Circulating antigen–antibody complexes are present and are probably responsible for the glomerulonephritis seen so often in this disease.

Delayed onset; recurrent fever; chronic disease; diarrhea

Severe systemic manifestations

Immune complex glomerulonephritis

## DIAGNOSIS AND TREATMENT

The diagnosis is made by demonstrating the presence of the organism in aspirates taken from the bone marrow, liver, spleen, or lymph nodes. In the Indian form of kala azar, *L. donovani* is also found in circulating monocytes. The specimens may be smeared, stained, and examined for the typical Leishman–Donovan bodies (amastigotes in mononuclear phagocytes) or cultured in artificial media and/or experimental animals. As described for cutaneous leishmaniasis, a limited number of reference laboratories can provide a rapid, direct, species-specific diagnosis through the use of the PCR and probes to kinetoplast DNA. Results of the leishmanin skin test are negative during active disease but become positive after successful therapy.

Demonstration of Leishman–Donovan bodies or culture

The mortality in untreated cases of kala azar is 75 to 90%. Treatment with pentavalent antimonial drugs lower this rate dramatically. Initial therapy, however, fails in up to 30% of African cases, and 15% of those that do respond eventually relapse. Resistant cases are treated with the more toxic pentamidine, amphotericin B, or liposomal amphotericin B. Allopurinol and interferon-$\gamma$ have proven to be useful adjunctive therapies in resistant cases. Control measures are directed at the *Phlebotomus* vector, with the use of residual insecticides, and at the elimination of mammalian reservoirs by treating human cases and destroying infective dogs.

Up to 90% mortality without treatment

# *African Trypanosoma*

## PARASITOLOGY

The trypanosomes that produce these diseases are morphologically and serologically identical. Accordingly, they are considered varieties of a single species, *Trypanosoma brucei.* The three subspecies, known as *T. brucei gambiense, T. brucei rhodesiense,* and *T. brucei brucei,* can be distinguished by their biologic characteristics, zymodeme types, mitochondrial morphology, and DNA hybridization patterns. All undergo similar developmental changes in the course of their passage between their insect and mammalian host. On ingestion by the tsetse fly (*Glossina* spp.), and after a period of multiplication in the midgut, they migrate to the insect's salivary glands and assume the epimastigote form. After a period of weeks they are transformed into metacyclic trypomastigotes, rendering them infectious to mammals. When the fly again takes a meal, the parasites are inoculated with the fly's saliva. In the mammalian host, they acquire a highly variable surface glycoprotein (VSG), multiply extracellularly, and eventually invade the bloodstream. During

Three recognized subspecies of *T. brucei*

Epimastigote and trypomastigote forms develop in tsetse fly the initial stages of parasitemia, some trypomastigotes elongate to become graceful, slender organisms 30 $\mu$m or more in length and divide every 5 to 10 hours. For reasons apparently independent of the host's immune response, multiplication eventually slows. Some forms lose their flagella and assume a short, stumpy appearance. The latter forms have a more developed mitochondria and are thought to be particularly infective to the insect host. Near the end of the episode of parasitemia, both morphologic types may be seen in a single blood specimen. Individual strains of *T. brucei* can change the antigenic character of their glycoprotein coat in a sequential and, at times, predictable fashion. A single strain is capable of producing dozens, perhaps hundreds, of these variable antigen types, each of which is encoded in its own structural gene. The genetic repertoire seems to be strain specific. Expression of individual genes appears to be controlled by the sequential duplication and subsequent transfer of each gene (expression-linked copy) to one or more areas of the genome responsible for gene expression.

Infectious trypomastigote form injected into the bloodstream of mammalian host from fly's saliva

Antigenic variation of glycoprotein coat of trypomastigotes is due to shifting expression of preexisting genes

## AFRICAN TRYPANOSOMIASIS (SLEEPING SICKNESS)

CLINICAL CAPSULE

African trypanosomiasis is a highly lethal meningoencephalitis transmitted to humans by bloodsucking flies of the genus *Glossina.* It occurs in two distinct clinical and epidemiologic forms: West African or Gambian sleeping sickness and East African or Rhodesian sleeping sickness. Nagana, a disease of cattle caused by a closely related trypanosome, renders over 10 million square kilometers of Central Africa unsuitable for animal husbandry.

### EPIDEMIOLOGY

The tsetse fly, and consequently sleeping sickness, is confined to the central area of Africa by that continent's two great deserts, the Sahara in the north and the Kalahari in the south. Approximately 50 million people live in this area and 10,000 to 20,000 acquire sleeping sickness annually. Major outbreaks have been reported in several locations within the endemic area over the past two decades, due, in part, to the internecine wars in this area that have interrupted control programs. Although an estimated 20,000 Americans travel to endemic areas each year, less than two dozen cases of African trypanosomiasis have been diagnosed in Americans since 1967.

Tsetse fly confined to central Africa

Riverine tsetse flies found in the forest galleries that border the streams of West and Central Africa serve as the vectors of the Gambian disease. Although these flies are not exclusively anthropophilic, humans are thought to be the major reservoir of the parasite. The infection rate in humans is affected by proximity to water but seldom exceeds 2 to 3% in nonepidemic situations. Nevertheless, the extreme chronicity of the human disease ensures its continued transmission.

Humans major reservoir of West African sleeping sickness; chronicity ensures maintenance

Rhodesian sleeping sickness, in contrast, is transmitted by flies indigenous to the great savannas of East Africa that feed on the blood of the small antelope inhabiting these areas. The antelope serves as the major parasite reservoir, although human-to-human and cattle-to-human spread has been documented. Humans typically become infected only when they enter the savanna to hunt or to graze their domestic animals. Currently, Sudan is the only country where both the Gambian and Rhodesian forms of the disease are still found. At present, there is little evidence of coinfections with African trypanosomes and HIV, possibly because the former is primarily rural in distribution and the latter is concentrated in cities.

Savanna antelopes are reservoirs of East African trypanosomiasis; humans infected incidentally

### PATHOGENESIS

Multiplication of the trypomastigotes at the inoculation site produces a localized inflammatory lesion. After the development of this chancre, organisms spread through

Local chancre at site of inoculation and lymphadenitis

Intermittent parasitemia with antigenic shifts

Parasites localize in blood vessels of heart and CNS with local vasculitis

lymphatic channels to the bloodstream, inducing a proliferative enlargement of the lymph nodes. The subsequent parasitemia is typically low grade and recurrent. As host antibodies (predominantly IgM) are produced to the surface antigen characteristic of a particular parasitemic wave, they bind to the organism, leading to its destruction by lysis and opsonization. The trypomastigotes disappear from the blood, reappearing 3 to 8 days later as new antigenic variants arise. The recurrences gradually become less regular and frequent but may persist for weeks to years before finally disappearing. During the course of the parasitemia, trypanosomes localize in the small blood vessels of the heart and central nervous system (CNS). This localization results in endothelial proliferation and a perivascular infiltration of plasma cells and lymphocytes. In the brain, hemorrhage and a demyelinating panencephalitis may follow.

High levels of IgM include specific and nonspecific antibodies

Immune complexes may cause anemia and vasculitis

The mechanism by which the trypanosomes elicit vasculitis is uncertain. The infection stimulates a massive, nonspecific polyclonal activation of B cells, the production of large quantities of immunoglobulin M (typically 8 to 16 times the normal limit) and the suppression of other immune responses. Most of this reaction represents specific protective antibodies that are ultimately responsible for the control of the parasitemia. Some, however, consists of nonspecific heterophile antibodies, antibodies to DNA, and rheumatoid factor. Antibody-induced destruction of trypanosomes releases invariant nuclear and cytoplasmic antigens with the production of circulating immune complexes. Many authorities believe that these complexes are largely responsible for the anemia and vasculitis seen in this disease.

# AFRICAN TRYPANOSOMIASIS (SLEEPING SICKNESS): CLINICAL ASPECTS

## MANIFESTATIONS

Raised red papule on exposed surface

Parasitemic manifestations 2–3 weeks later

Late CNS involvement

The trypanosomal chancre appears 2 to 3 days after the bite of the tsetse fly as a raised, reddened nodule on one of the exposed surfaces of the body. With the onset of parasitemia 2 to 3 weeks later, the patient develops recurrent bouts of fever, tender lymphadenopathy, skin rash, headache, and impaired mentation. In the Rhodesian form of disease, myocarditis and CNS involvement begin within 3 to 6 weeks. Heart failure, convulsions, coma, and death follow in 6 to 9 months. Gambian sleeping sickness progresses more slowly. Bouts of fever often persist for years before CNS manifestations gradually appear. Spontaneous activity progressively diminishes, attention wavers, and the patient must be prodded to eat or talk. Speech grows indistinct, tremors develop, sphincter control is lost, and seizures with transient bouts of paralysis occur. In the terminal stage, the patient develops a lethal intercurrent infection or lapses into a final coma.

## DIAGNOSIS

Trypomastigotes sought in lymph node aspirates, blood, and cerebrospinal fluid

Animal inoculation may be required in Rhodesian disease

A definitive diagnosis is made by microscopically examining lymph node aspirates, blood, or cerebrospinal fluid for the presence of trypomastigotes. Early in the disease, actively motile organisms can often be seen in a simple wet mount preparation coat smear; identification requires examination of an appropriately stained smear. If these tests prove negative, the blood can be centrifuged and the stained buffy coat examined. Inoculation of rats or mice can also prove helpful in diagnosing the Rhodesian disease. The patient may also be screened for elevated levels of IgM in the blood and spinal fluid or specific trypanosomal antibodies by a variety of techniques. A simple card agglutination test, which can be performed on finger-stick blood, can provide serologic confirmation within minutes. Subspecies-specific DNA probes may eventually prove useful for the identification of organisms in clinical specimens.

## TREATMENT

Lumbar puncture must always be performed before initiation of therapy. If the specimen reveals evidence of CNS involvement, agents that penetrate the blood–brain barrier must be included. Unfortunately, the most effective agent of this type is a highly toxic arsenical, melarsoprol (Mel B). Although this agent occasionally produces a lethal hemorrhagic encephalopathy, the invariably fatal outcome of untreated CNS disease warrants its use. The ornithine decarboxylase inhibitor, eflornithine appears capable, when used alone, or together with suramin, of curing CNS disease caused by *T. brucei gambiense* without the serious side effects associated with melarsoprol. Unfortunately, it is very expensive and is only variably effective in *T. brucei rhodesiense* infections. If the CNS is not yet involved, less toxic agents, such as suramin, pentamidine, or eflornithine, can be used. In such cases, the cure rate is high and recovery complete.

Selection of drugs dependent on whether CNS is involved

Without CNS involvement, recovery often complete

## PREVENTION

Although a variety of tsetse fly control measures, including the use of insecticides, deforestation, and the introduction of sterile males into the fly population, have been attempted, none has proved totally practicable. Similarly, eradication of disease reservoirs by the early detection and treatment of human cases and the destruction of wild game has had limited success. Attempts to develop effective vaccines are currently under way but are complicated by the antigenic variability of most trypomastigotes. A degree of personal protection can be achieved with insect repellents and protective clothing. Although prophylactic use of pentamidine was once advocated, enthusiasm for this treatment has waned.

Neither vector or reservoir control has been successful

# *American Trypanosoma*

## PARASITOLOGY

The trypomastigotes of *Trypanosoma cruzi* closely resemble those of *T. brucei,* and like them, disseminate from the site of inoculation to circulate in the peripheral blood of their mammalian hosts. Their developmental cycle, however, differs in several respects. Most significant, *T. cruzi* does not multiply extracellularly. The circulating trypomastigotes must invade tissue cells, lose their flagella, and assume the amastigote form before binary fission can occur. Continued multiplication leads to distention and eventual rupture of the tissue cell. Released parasites revert to trypomastigotes and regain the bloodstream. This new generation of trypomastigotes may invade other host cells, thus continuing the mammalian cycle. Alternatively, they may be ingested by a feeding reduviid and develop into epimastigotes within its midgut. On completion of the invertebrate cycle, the parasites migrate to the hindgut and are discharged as infectious trypomastigotes when the reduviid defecates in the process of taking another blood meal. This process can recur at each feeding for as long as 2 years. Infection in the new host is initiated when the trypomastigotes contaminate either the feeding site or the mucous membranes.

Mammalian cycle with nondividing extracellular trypomastigotes and dividing intracellular amastigotes

Invertebrate cycle produces trypomastigotes in bug

Reduviid bug may remain infectious for up to 2 years

*T. cruzi* comprises a number of strains, each with its own distinct geographic distribution, tissue preference, and virulence. They may be distinguished from one another with specific antisera and by differences in their isoenzyme and DNA restriction patterns. All are morphologically identical. In blood specimens, the trypomastigotes can be distinguished from those of *T. brucei* by their characteristic C or U shape, narrow undulating membrane, and large kinetoplast.

# AMERICAN TRYPANOSOMIASIS (CHAGAS' DISEASE)

CLINICAL CAPSULE

American trypanosomiasis is a disease produced by *T. cruzi* and transmitted by true bugs of the family Reduviidae. Clinically, the infection presents as an acute febrile illness in children and a chronic heart or gastrointestinal malady in adults.

## EPIDEMIOLOGY

Chagas' disease in South and Central America

"Kissing bug" feeds at night in rural areas

Chagas' disease affects 16 to 18 million people in a geographic area extending from Central America to southern Argentina, producing death in 50,000 annually. Within these areas, it is the leading cause of heart disease, accounting for one fourth of all deaths in the 25- to 44-year age group. Transmission occurs primarily in rural settings where the reduviid can find harborage in animal burrows and in the cracked walls and thatch of poorly constructed buildings. This large (3-cm), winged insect leaves its hiding place at night to feed on its sleeping hosts. Its predilection to bite near the eyes or lips have earned this pest the nicknames of "kissing bug" and "assassin bug." Most new infections in these areas occur in children. Infection can also be acquired in utero, and, less frequently, through breastfeeding.

Other wild and domestic animal reservoirs amplify transmission

In addition to humans, a number of wild and domestic animals, including rats, cats, dogs, opossums, and armadillos, serve as reservoirs. The close association of many of these hosts with human dwellings tends to amplify the incidence of disease in humans and the difficulty involved in its control.

Organ transplantation and transfusion-related infections are rapidly increasing problems in urban settings within endemic areas. Recrudescence of the latent infection is increasingly seen in immunosuppressed individuals, including patients with HIV infections. More effective blood bank screening provides hope that transmission of this disease will be substantially curtailed in the near future.

An estimated 50,000 infected Latin American immigrants are currently living in the United States. Because *T. cruzi* has been found in both vertebrate and invertebrate hosts in the southwestern United States, there is a possibility of sustained transmission of this organism within this country. Although serologic evidence suggests that the acquisition of human infection in this area is not uncommon, clinically apparent autochthonous cases have been rare. The majority of these acquired the infection through blood–blood transfusions.

## PATHOGENESIS

Local chancre at site of inoculation

Entry to mesenchymal cells facilitated by fibronectin binding surface protein

Pore-forming protein aids escape from phagolysosome

Pseudocysts formed from cytoplasmic multiplication in host cells

Multiplication of the parasite at the portal of entry stimulates the accumulation of neutrophils, lymphocytes, and tissue fluid, resulting in the formation of a local chancre or chagoma. The subsequent dissemination of the organism with invasion of tissue cells produces a febrile illness that may persist for 1 to 3 months and result in widespread organ damage. Any nucleated host cell may be involved but those of mesenchymal origin, especially the heart, skeletal muscle, smooth muscle, and glial nerve cells, are particularly susceptible. Cell entry is facilitated by binding to host cell fibronectin; a 60-kd *T. cruzi* surface protein (penetrin) appears to promote adhesion. Following penetration, the trypomastigote escapes the phagolysosome via the production of a pore-forming protein, transforms to the amastigote form, and multiplies freely within the cytoplasm to produce a pseudocyst, a greatly enlarged and distorted host cell containing masses of organisms. With the rupture of the pseudocyst, many of the released parasites disintegrate, eliciting an intense inflammatory reaction with destruction of surrounding tissue. The development of an antibody-dependent, cell-mediated immune response leads to the eventual destruction of the *T. cruzi* parasites and the termination of the acute phase of illness.

Parasitic antigens released during this acute phase may bind to the surface of tissue cells, rendering them susceptible to destruction by the host's immune response. It has been suggested by some that this results in the production of antibodies that cross-react with host tissue, initiating a sustained autoimmune inflammatory reaction in the absence of systemic manifestation of illness. In the heart, this reaction leads to changes in coronary microvasculature, loss of muscle tissue, interstitial fibrosis, degenerative changes in the myocardial conduction system, and loss of intracardiac ganglia. In the digestive tract, loss of both ganglionic nerve cells and smooth muscle results in dilatation and loss of peristaltic movement, particularly of the esophagus and colon.

Damage to heart may have immune mechanism

Ganglionic and smooth muscle cells lost in digestive tract

# AMERICAN TRYPANOSOMIASIS (CHAGAS' DISEASE): CLINICAL ASPECTS

## MANIFESTATIONS

Serologic studies suggest that only one third of newly infected individuals develop clinical illness. Acute manifestations, when they occur, are seen primarily in children. They begin with the appearance of the nodular, erythematous chagoma 1 to 3 weeks after the bite of the reduviid. If the eye served as a portal of entry, the patient will present with Romaña's sign: reddened eye, swollen lid, and enlarged preauricular lymph node. The onset of parasitemia is signaled by the development of a sustained fever; enlargement of the liver, spleen, and lymph nodes; signs of meningeal irritation; and the appearance of peripheral edema or a transient skin rash. In a small percentage of symptomatic patients, heart involvement results in tachycardia, electrocardiographic changes, and occasionally arrhythmia, enlargement, and congestive heart failure. Newborns may experience acute meningoencephalitis. Clinical manifestations persist for weeks to months. In 5 to 10% of untreated patients, severe myocardial involvement or meningoencephalitis leads to death.

Most infections asymptomatic; acute disease usually in children

Myocardial injury indicated by tachycardia and electrocardiographic changes

Chronic disease, the result of end-stage organ damage, is usually seen only in adulthood. Ironically, the majority of patients with late manifestations deny a history of acute illness. The most serious of the late manifestations is heart disease. Studies of asymptomatic, seropositive patients in endemic areas have shown that a significant proportion have cardiac abnormalities demonstrated by electrocardiographic, echocardiographic, or cineangiographic techniques, suggesting that Chagas' cardiomyopathy is a progressive, focal disease of the myocardium and conduction system, leading eventually to clinical disease. This may present as arrhythmia, thromboembolic events, heart block, enlargement with congestive heart failure, and cardiac arrest. In some areas of rural Latin America, as much as 10% of the adult population may show cardiac manifestations. In the United States, chagasic heart disease in immigrants is usually initially misdiagnosed as coronary artery disease or idiopathic dilated cardiomyopathy. Megaesophagus and megacolon, which are less devastating than the heart disease, are typically seen in more southern latitudes. This geographic variation in clinical manifestations is thought to be attributable to a difference in tissue tropism between individual strains of *T. cruzi.* Megaesophagus leads to difficulty in swallowing and regurgitation, particularly at night. Megacolon produces severe constipation with irregular passage of voluminous stools. *T. cruzi* brain abscess has been described in a small number of AIDS patients.

Chronic cardiomyopathy in adults leads to heart block and/or congestive heart failure

Dilatation of esophagus and colon seen in southern latitudes

## DIAGNOSIS

The diagnosis of acute Chagas' disease rests on finding the trypomastigotes in the peripheral blood or buffy coat, and their morphologic identification as *T. cruzi.* The methods are similar to those described for diagnosis of African trypanosomiasis. If the results are negative, a laboratory-raised reduviid can be fed on the patient, then dissected and examined for the presence of parasites, a procedure known as xenodiagnosis. Alternatively, the blood may be cultured in a variety of artificial media or experimental animals. In the diagnosis of chronic disease, recovery of the organisms is the exception rather than the rule, and diagnosis depends on the clinical, epidemiologic, and immunodiagnostic findings.

Demonstration of trypomastigotes in peripheral blood

Xenodiagnosis involves allowing bugs to feed

Organisms difficult to recover in chronic disease

A variety of serologic tests are available; small numbers of false-positive results limit their usefulness, particularly when used as screening procedures in non-endemic areas. The recent production of specific recombinant proteins and synthetic peptides for use as antibody targets may improve the reliability of these procedures. PCR techniques for the amplification of trypomastigote DNA are available in a small number of research laboratories.

## TREATMENT

Treatment may reduce acute disease

The role of treatment in Chagas' disease remains unsettled. Two agents, nifurtimox and benznidazole, effectively reduce the severity of acute disease but appear to be ineffective in chronic infections. Both drugs must be taken for prolonged periods of time, may cause serious side effects and do not always result in parasitologic cure. Allopurinol, a hypoxanthine oxidase inhibitor devoid of serious side effects, has recently been shown to be capable of suppressing parasitemia and reversing the serostatus of patients with acute disease. Additional studies to confirm these encouraging results are necessary.

## PREVENTION

Control of reduviid bugs in rural homes most important measure

The reduviid vector can be controlled by applying residual insecticides to rural buildings at 2- or 3-month intervals. The addition of latex to the insecticide creates a colorless paint that prolongs activity. Fumigants can be used to prevent reinfection. Patching wall cracks, cementing floors, and moving debris and woodpiles away from human dwellings reduces the number of reduviids within the home. Transfusion-induced disease, a major problem in endemic areas, has been partially controlled by the addition of gentian violet to all blood packs before use or by screening potential donors serologically for Chagas' disease. The large number of infected immigrants now entering nonendemic countries presents an increasing risk of transfusion-mediated parasite transmission in these areas as well. Cases of acute Chagas' disease have been reported in the United States in immunosuppressed patients who received blood from donors unaware of their infection status; the resulting diseases were particularly fulminant. Immunodiagnostic tests for Chagas' disease are neither readily available nor sufficiently specific for use in nonendemic areas; prevention will probably require deferral of blood donations from persons who have recently immigrated from endemic areas. Immunoprophylaxis is not available at present.

# ADDITIONAL READING

Adam RD. Biology of *Giardia lamblia. Clin Microbiol Rev* 2001;14:447–475. A detailed review of the biology and taxonomy of this pathogen, which leads the author to propose that the major strains infecting humans warrant separate species or subspecies designations.

Adler S. Darwin's illness. *Nature* (London) 1959;184:1102–1103. The author describes Charles Darwin's 40-year illness and offers convincing arguments that it represented Chagas' disease acquired during Darwin's round-the-world expedition on *H.M.S. Beagle.*

Berman JD. Human leishmaniasis: clinical, diagnostic and chemotherapeutic developments in the last 10 years. *Clin Infect Dis* 1997;24:684–703.

Burri C, Nkunku S, Merolle A, et al. Efficacy of new, concise schedule for melarsoprol in treatment of sleeping sickness caused by *Trypanosoma brucei gambiense*: a randomized trial. *Lancet* 2000;355:1419–1425.

Gardner TB, Hill DR. Treatment of giardiasis. *Clin Microbiol Rev* 2001;14:114–128. A recent update.

Legros D, et al. Treatment of human African trypanosomiasis—present situation and needs for research and development. *Lancet (Infect Dis)* 2002;2:437–440.

Leiby DA, Read EJ, Lenes BA, et al. Seroepidemiology of *Trypanosoma cruzi,* etiologic agent of Chagas' disease, in U.S. blood donors. *J Infect Dis* 1997;176:1047–1052.

Petrin D, Delgaty K, Bhatt R, Garber G. Clinical and microbiologic aspects of *Trichomonas vaginalis. Clin Microbiol Rev* 1998;11:300–317.

Prata A. Clinical and epidemiological aspects of Chagas disease. *Lancet (Infect Dis)* 2001;1:92–100. An excellent, brief current review of this fascinating, debilitating New World disease.

Warren KS (ed). *Immunology and Molecular Biology of Parasitic Infections,* 3rd ed. Boston: Blackwell Scientific; 1993. This relatively comprehensive monograph discusses general immune responses to parasitic infections as well as the immunity, immunopathology, immunodiagnosis, and molecular biology of specific parasitic diseases.

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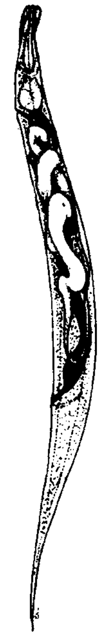
CHAPTER 55

# Intestinal Nematodes

JAMES J. PLORDE



Nematode

The intestinal nematodes have cylindric, fusiform bodies covered with a tough, acellular cuticle. Sandwiched between this integument and the body cavity are layers of muscle, longitudinal nerve trunks, and an excretory system. A tubular alimentary tract consisting of a mouth, esophagus, midgut, and anus runs from the anterior to the posterior extremity. Highly developed reproductive organs fill the remainder of the body cavity. The sexes are separate; the male worm is generally smaller than its mate. The female, which is extremely prolific, can produce thousands of offspring, generally in the form of eggs. Typically, the eggs must incubate or embryonate outside of the human host before they become infectious to another person; during this time, the embryo repeatedly segments, eventually developing into an adolescent form known as a larva. In some species of nematodes, offspring develop to the larval stage in the uterus of the worm. The duration and site of embryonation differ with each worm species and determine how it will be transmitted to the new host. In many cases, eggs of nematodes that dwell within the human gastrointestinal tract are carried to the environment in the feces and embryonate on the soil for a period of weeks before becoming infectious. The egg may then be ingested with contaminated food. In some species, the egg hatches outside of the host, releasing a larva capable of penetrating the skin of a person who comes in direct physical contact with it. Obviously, intestinal nematodes are principally found in areas where human feces are deposited indiscriminately or used for fertilizer.

Six intestinal nematodes commonly infect humans: *Enterobius vermicularis* (pinworm), *Trichuris trichiura* (whipworm), *Ascaris lumbricoides* (large roundworm), *Necator americanus* and *Ancylostoma duodenale* (hookworms), and *Strongyloides stercoralis.* Together they infect more than one fourth of the human race, producing embarrassment, discomfort, malnutrition, anemia, and occasionally death. Other closely related nematodes of animals that may occasionally infect humans are also listed in Table 55–1, but will not be discussed here.

Long survival in gut lumen

Worm load and repeated infection important to disease severity

The adults of each of the six nematodes listed previously can survive for months or years within the lumen of the gut. The severity of illness produced by each depends on the level of adaptation to the host it has achieved. Some species have a simple life cycle that can be completed without serious consequences to the host. Less well-adapted parasites, on the other hand, have more complex cycles, often requiring tissue invasion and/or production of enormous numbers of offspring to ensure their continued survival and dissemination. Within a given species, disease severity is related directly to the number of adult worms harbored by the host. The greater the worm load or worm burden, the more serious the consequences. Because nematodes do not multiply within the human, small worm loads may remain asymptomatic and undetected throughout the lifespan of the parasite. Repeated infections, however, progressively increase the worm burden and at some

TABLE 55–1

**Intestinal Nematodes**

| Human Parasite | Animal Parasite | Human Disease |
|---|---|---|
| *Enterobius vermicularis* (pinworm) | | Enterobiasis |
| *Trichuris trichiura* (whipworm) | | Trichuriasis |
| | *Capillaria philippinensis* | Intestinal capillariasis |
| *Ascaris lumbricoides* (large roundworm) | | Ascariasis |
| | *Ascaris suum* | Ascariasis |
| | *Anisakis* spp. | Anisakiasis |
| | *Toxocara canis* | Toxocariasis (visceral larva migrans) |
| | *Toxocara cati* | |
| *Necator americanus* (hookworm) | | Hookworm disease |
| *Ancylostoma duodenale* (hookworm) | *Ancylostoma braziliense* | Cutaneous larva migrans |
| *Strongyloides stercoralis* | | Strongyloidiasis |

point induce symptomatic disease. Although humans can mount an immune response that will eventually lead to the expulsion of worms, it is slow to develop and incomplete. It is therefore the frequency and intensity of reinfection, more than the host's immune response, that determine the worm burden. This burden is seldom uniform within affected populations, but rather "aggregated" within subgroups related to their hygienic practices.

## Life Cycles

The life cycles of the intestinal nematodes are summarized in Table 55–2. *E. vermicularis* (pinworm), the best adapted of the intestinal nematodes, has the simplest life cycle. It feeds, grows, and copulates within the gut of its host before transiting the anus to deposit its eggs on the perineal skin. The eggs embryonate within hours and are subsequently transported to the same, or a new, host via fingers or dust. Following their inhalation or ingestion, the eggs are swallowed and hatch in the bowel lumen, completing the cycle. The only significant difference between this and the life cycle of *T. trichiura* (whipworm) is that the eggs of the latter are passed in the stool and must incubate on soil before becoming infectious. This relatively minor difference has profound epidemiologic ramifications, because *Trichuris* can be passed only in populations that practice indiscriminate defecation and live in climates suitable for the maturation of eggs in the soil.

*Enterobius vermicularis* is the best adapted intestinal nematode

*A. lumbricoides* is transmitted in a manner similar to *T. trichiura.* However, after hatching from the egg in the gut lumen, ascarid larvae penetrate the bowel wall and migrate through the host's liver and lung before returning, older and more sedentary, to the protective environment of the gut lumen. This maladaptive sojourn of juvenile worms through the host tissue is also seen in the life cycles of the hookworms and *S. stercoralis.* In contrast to *Ascaris,* however, the eggs of the latter two nematodes hatch shortly before or after they are passed in the stool of the original host, resulting in the seeding of the external environment with larval forms capable of penetrating human skin. Transmission is effected when a new host comes into physical contact with the contaminated soil. The adaptation of *S. stercoralis* is the least satisfactory of the intestinal nematodes and, in an evolutionary sense, appears to have occurred quite recently. In addition to the hookworm-like cycle described above, it has the twin capacities to complete its life cycle

Other nematodes have increasingly complex life cycles

*S. stercoralis* is least well adapted

TABLE 55–2

**Life Cycles of Intestinal Nematodes**

| Parasite | Route of Infection | Migration in Body | Diagnostic Form | Site of Embryonation | Infective Form | Free-Living Cycle |
|---|---|---|---|---|---|---|
| *Enterobius vermicularis* | Mouth | Intestinal | Egg | Perineum | Egg | No |
| *Trichuris trichiura* | Mouth | Intestinal | Egg | Soil | Egg | No |
| *Ascaris lumbricoides* | Mouth | Pulmonary | Egg | Soil | Egg | No |
| *Necator americanus*[a] | Skin | Pulmonary | Egg | Soil | Filariform larvae | No |
| *Stronglyoides stercoralis* | Skin | Pulmonary | Rhabditiform larvae | Soil; intestine[b] | Filariform larvae | Yes |

Reproduced with permission from Plorde JJ. In Isselbacher KJ, et al: *Harrison's Principles of Internal Medicine,* 9th ed. New York, McGraw-Hill, 1980, Table 206–3, p. 891.

[a]Also *Ancylostoma duodenale*.

[b]Intestine only in cases of autoinfection.

entirely within the body of the host or to survive in the external environment as a free-living soil organism.

# PARASITES AND DISEASES

## *Enterobius*

### *Enterobius vermicularis* (PINWORM): PARASITOLOGY

The adult female is a 10-mm-long, cream-colored worm with a sharply pointed tail, characteristics that have given rise to the common name pinworm. Running longitudinally down both sides of the body are small ridges that widen anteriorly to fin-like alae. The seldom-seen male is smaller (3 mm) and possesses a ventrally curved tail and copulatory spicule. The clear, thin-shelled, ovoid eggs are flattened on one side and measure 25 by 50 $\mu$m (Fig 55–1).

Common name is pinworm

#### LIFE CYCLE

Adults inhabit cecum

The adult worms lie attached to the mucosa of the cecum. As its period of gravidity draws to a close, the female migrates down the colon, slips unobserved through the anal canal in the dark of the night, and deposits as many as 20,000 sticky eggs on the host's perianal skin, bedclothes, and linens. The eggs are near maturity at the time of deposition and become infectious shortly thereafter. Handling of bedclothes or scratching of the perianal area to relieve the associated itching results in adhesion of the eggs to the fingers and subsequent transfer to the oral cavity during eating or other finger–mouth maneuvers. Alternatively, the eggs may be shaken into the air (eg, during making of the bed), inhaled, and swallowed. The eggs subsequently hatch in the upper intestine and the larvae migrate

Female transits anus at night to deposit eggs on perineum

Eggs infectious to host and others shortly after deposition

Ingested eggs hatch and larvae mature to adults in intestine

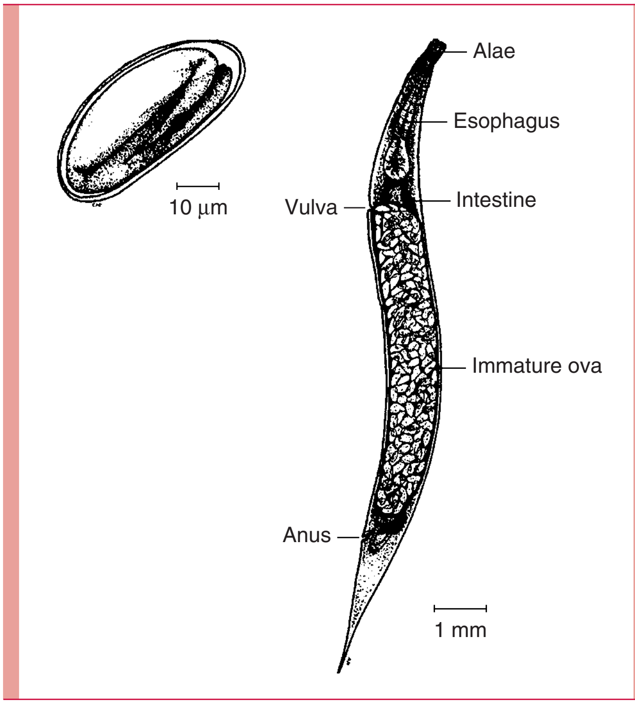



FIGURE 55–1
Female pinworm (*Enterobius vermicularis*) and embryonated egg.

to the cecum, maturing to adults and mating in the process. The entire adult-to-adult cycle is completed in 2 weeks.

# ENTEROBIASIS

## EPIDEMIOLOGY

Infects 30–40 million in United States

The pinworm is the oldest and most widespread of the helminths. Eggs have been found in a 10,000-year-old coprolith, making this nematode the oldest demonstrated infectious agent of humans. It has been estimated to infect at least 200 million people, particularly children, worldwide, and 40 million in the United States alone. Despite evidence that its prevalence is now decreasing in the United States, in both that country and in western Europe it remains the single most common cause of human helminthiasis. Infection is more common among the young and poor, but may be found in any age or economic class.

Resistant infective eggs

The eggs are relatively resistant to desiccation and may remain viable in linens, bedclothes, or house dust for several days. Once infection is introduced into a household, other family members are rapidly infected.

## PATHOGENESIS AND IMMUNITY

The adult worms produce no significant intestinal pathology and do not appear to induce protective immunity.

# ENTEROBIASIS: CLINICAL ASPECTS

## MANIFESTATIONS

*E. vermicularis* seldom produces serious disease. The most frequent symptom is pruritus ani (anal itching). This symptom is most severe at night and has been attributed to the migration of the gravid female. It may lead to irritability and other minor complaints. In severe infections, the intense itching may lead to scratching, excoriation, and secondary bacterial infection. In female patients, the worm may enter the genital tract, producing vaginitis, granulomatous endometritis, or even salpingitis. It has also been suggested that migrating worms might carry enteric bacteria into the urinary bladder in young women, inducing an acute bacterial infection of the urinary tract. Although this worm is frequently found in the lumen of the resected appendix, it is doubtful that it plays a causal role in appendicitis. Perhaps the most serious effect of this common infection is the psychic trauma suffered by the economically advantaged when they discover that they, too, are subject to intestinal worm infection.

Nocturnal pruritus ani

Occasional infection of female genitourinary tract

## DIAGNOSIS

Eosinophilia is usually absent. The diagnosis is suggested by the clinical manifestations and confirmed by the recovery of the characteristic eggs from the anal mucosa. Identification is accomplished by applying the sticky side of cellophane tape to the mucocutaneous junction, then transferring the tape to a glass slide and examining the slide under the low-power lens of a microscope. Occasionally, the adult female is seen by a parent of an infected child or recovered with the cellophane tape procedure.

Anal cellophane tape test detects ova

## TREATMENT AND PREVENTION

Several highly satisfactory agents, including pyrantel pamoate and mebendazole, are available for treatment. Many authorities believe that all members of a family or other cohabiting group should be treated simultaneously. In severe infections, retreatment after 2 weeks is recommended. Although cure rates are high, reinfection is extremely common. It need not be treated in the absence of symptoms.

All family members may need treatment

Reinfection common

# *Trichuris*

## *Trichuris trichiura* (WHIPWORM): PARASITOLOGY

The adult whipworm is 30 to 50 mm in length. The anterior two thirds is thin and threadlike, whereas the posterior end is bulbous, giving the worm the appearance of a tiny whip. The tail of the male is coiled; that of the female is straight. The female produces 3000 to 10,000 oval eggs each day. They are of the same size as pinworm eggs but have a distinctive thick brown shell with translucent knobs on both ends (Fig 55–2).

Whipworm produces up to 10,000 eggs a day

## LIFE CYCLE

*Trichuris trichiura* has a life cycle that differs from that of the pinworm only in its external phase. The adults live attached to the colonic mucosa by their thin anterior end. While retaining its position in the cecum, the gravid female releases its eggs into the lumen of the gut. These pass out of the body with the feces and, in poorly sanitated areas of the world, are deposited on soil. The eggs are immature at the time of passage and must incubate for at least 10 days (longer if soil conditions, temperature, and moisture are suboptimal) before they become fully embryonated and infectious. Once mature, they are picked up on the hands of children at play or of agricultural workers and passed to the mouth. In areas where human feces are used as fertilizer, raw fruits and vegetables may be contaminated and later ingested. Following ingestion, the eggs hatch in the duodenum, and the released larvae mature for approximately 1 month in the small bowel before migrating to their adult habitat in the cecum.

Adults inhabit cecum and release eggs to lumen

Eggs must mature in soil for 10 days

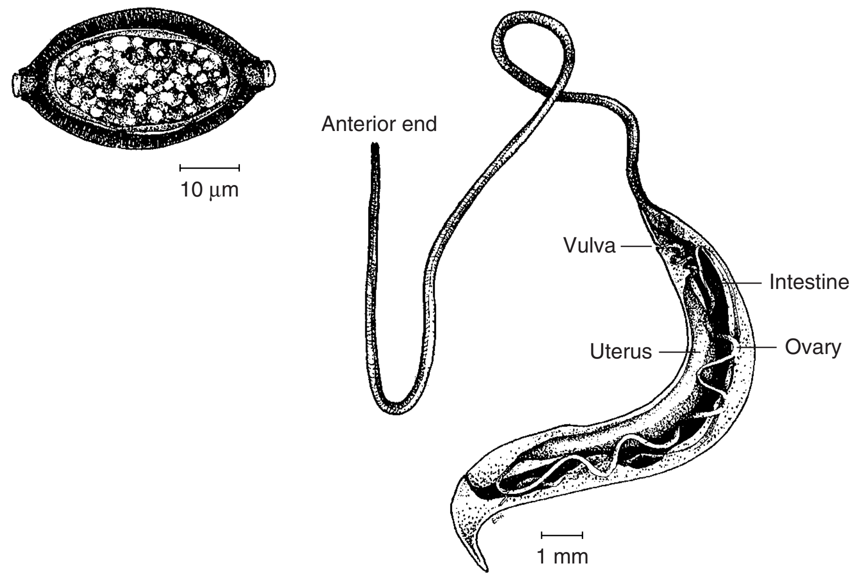



FIGURE 55–2
Female whipworm (*Trichuris trichiura*) and embryonated egg.

# TRICHURIASIS

## EPIDEMIOLOGY

Associated with defecation on soil and warm, humid climate

Adult worms live for years

Although it is less widespread than the pinworm, the whipworm is a cosmopolitan parasite, infecting approximately 1 billion people throughout the world. It is concentrated in areas where indiscriminate defecation and a warm, humid environment produce extensive seeding of soil with infectious eggs. In tropical climates, infection rates may be as high as 80%. Although the incidence is much lower in temperate climates, trichuriasis affects 2 million individuals throughout the rural areas of the southeastern United States. Here it occurs primarily in family and institutional clusters, presumably maintained by the poor sanitary habits of toddlers and the mentally retarded. Although the intensity of infection is generally low, adult worms may live 4 to 8 years.

## PATHOGENESIS AND IMMUNITY

Local colonic ulceration provides entry point to bloodstream for bacteria

Attachment of adult worms to the colonic mucosa and their subsequent feeding activities produce localized ulceration and hemorrhage (0.005 mL blood per worm per day). The ulcers provide enteric bacteria with a portal of entry to the bloodstream, and occasionally a sustained bacteremia results. A decrease in the prevalence of trichuriasis in the postadolescent period and the demonstration of acquired immunity in experimental animal infections suggest that immunity may develop in naturally acquired human infections. An IgE-mediated immune mucosal response is demonstrable in humans, but is insufficient to cause appreciable parasite expulsion.

# TRICHURIASIS: CLINICAL ASPECTS

## MANIFESTATIONS

Light infections are asymptomatic. With moderate worm loads, damage to the intestinal mucosa may induce nausea, abdominal pain, diarrhea, and stunting of growth. Occasionally, a child may harbor 800 worms or more. In these situations, the entire colonic mucosa is parasitized, with significant mucosal damage, blood loss, and anemia. The shear force of the fecal stream on the bodies of the worms may produce prolapse of the colonic or rectal mucosa through the anus, particularly when the host is straining at defecation or during childbirth.

Colonic damage with abdominal pain and diarrhea

Colonic or rectal prolapse with heavy worm load

## DIAGNOSIS

In light infections, stool concentration methods may be required to recover the eggs. Such procedures are almost never necessary in symptomatic infections, as they inevitably produce more than 10,000 eggs per gram of feces, a density readily detected by examining 1 to 2 mg of emulsified stool with the low-power lens of a microscope. A moderate eosinophilia is common in such infections.

Stools examined for characteristic eggs

## TREATMENT AND PREVENTION

Infections should not be treated unless they are symptomatic. Mebendazole is the drug of choice; albendazole is thought to be equally effective. Although the cure rate is only 60 to 70%, more than 90% of the adult worms are usually expelled, rendering the patient asymptomatic. Prevention requires the improvement of sanitary facilities.

# *Ascaris*

## *Ascaris lumbricoides*: PARASITOLOGY

*A. lumbricoides,* a short-lived worm (6 to 18 months), is the largest and most common of the intestinal helminths. Measuring 15 to 40 cm in length, it dwarfs its fellow gut roundworms and brings an unexpected richness to our mental image of a parasite. Its firm, creamy cuticle and more pointed extremities differentiate it from the common earthworm, which it otherwise resembles in both size and external morphology. The male is slightly smaller than the female and possesses a curved tail with copulatory spicules. The female passes 200,000 eggs daily, whether or not she is fertilized. Eggs are elliptic in shape; measure 35 by 55 $\mu$m; and have a rough, mamillated, albuminous coat over their chitinous shells. They are highly resistant to environmental conditions and may remain viable for up to 6 years in mild climates (Fig 55–3).

Earthworm-sized roundworm produces elliptical eggs

Eggs viable up to 6 years

## LIFE CYCLE

The adult ascarids live high in the small intestine, where they actively maintain themselves by dint of muscular activity. The eggs are deposited into the intestinal lumen and passed in the feces. Like those of *Trichuris,* the eggs must embryonate in soil, usually for a minimum of 3 weeks, before becoming infectious. The similarity to *Trichuris* ends, however, with the ingestion of the eggs by the host. After hatching, the larvae penetrate the intestinal mucosa and invade the portal venules. They are carried to the liver, where they are still small enough to squeeze through that organ's capillaries and exit in the hepatic vein. They are then carried to the right side of the heart and subsequently pumped out to the lung. In the course of this migration, the larvae increase in size. By the time they reach the pulmonary capillaries, they are too large to pass through to the left side of the heart. Finding their route blocked, they rupture into the alveolar spaces, are coughed up, and subsequently swallowed. After regaining access to the upper intestine, they complete their maturation and mate.

Adults inhabit small intestine

Eggs must mature for 3 weeks in soil

Larvae from ingested eggs enter bloodstream and pass through alveoli and via respiratory tract and esophagus to intestines

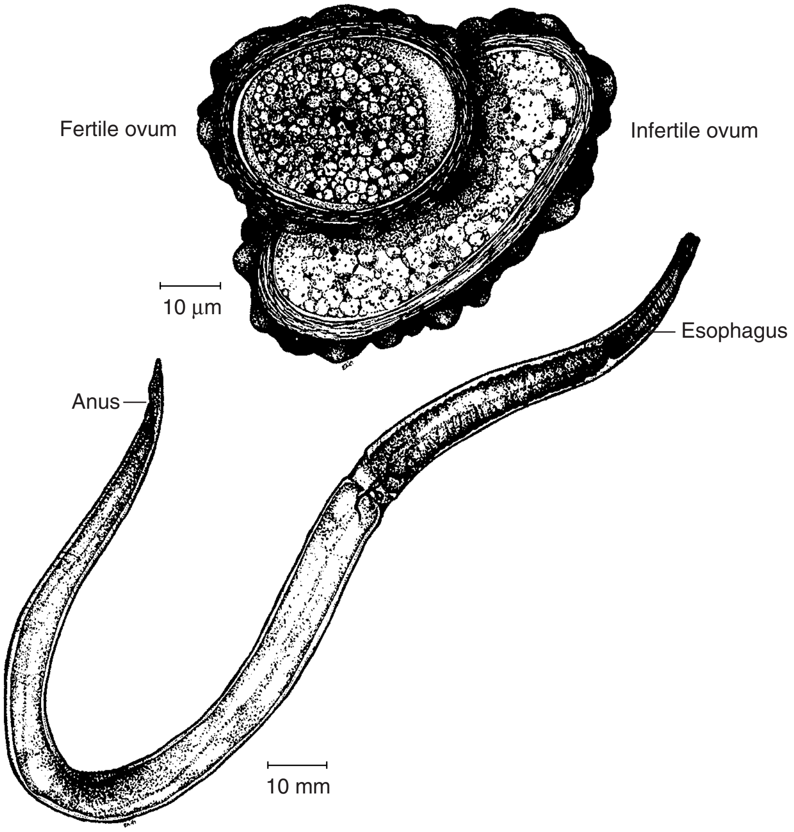



FIGURE 55–3
Female *Ascaris lumbricoide*s worm and fertile and infertile egg.

# ASCARIASIS

## EPIDEMIOLOGY

Epidemiology similar to that of *Trichuris*

More than 1 billion of the world's population, including 4 million Americans, are infected. Together they have been estimated to pass more than 25,000 tons of *Ascaris* eggs into the environment annually. Like trichuriasis, with which it is coextensive, ascariasis is a disease of warm climates and poor sanitation. It is maintained by small children who defecate indiscriminately in the immediate vicinity of the home and pick up infectious eggs on their hands during play. Geophagia may result in massive worm loads. The parasite may also be acquired through ingestion of egg-contaminated food by the host; in dry, windy climates, eggs may become airborne and be inhaled and swallowed. In tropical areas, the entire population may be involved; most worms, however, appear to be aggregated in a minority of the population, suggesting that some individuals are predisposed to heavy infections. Isolated infected family clusters are more common in temperature climates.

## PATHOGENESIS AND IMMUNITY

Hypersensitive pulmonary reactions to larval migration

There is convincing evidence that ascariasis induces a protective immune response in the host. Moreover, the severity of pulmonary damage induced by the migration of larvae through the lung appears to be related in part to an immediate hypersensitivity reaction to larval antigens.

## ASCARIASIS: CLINICAL ASPECTS

### MANIFESTATIONS

Clinical manifestations may result from either the migration of the larvae through the lung or the presence of the adults in the intestinal lumen. Pulmonary involvement is usually seen in communities where transmission is seasonal; the severity of symptoms is related to the degree of hypersensitivity induced by previous infections and the intensity of the current exposure. Fever, cough, wheezing, and shortness of breath are common. Laboratory studies reveal eosinophilia, oxygen denaturation, and migratory pulmonary infiltrates. Death from respiratory failure has been noted occasionally.

If the worm load is small, infections with adult worms may be completely asymptomatic. They come to clinical attention when the parasite is vomited up or passed in the stool. This situation is most likely during episodes of fever, which appear to stimulate the worms to increase motility. Most physicians who have worked in developing countries have had the disconcerting experience of observing an ascarid crawl out of a patient's mouth, nose, or ear during an otherwise uneventful evaluation of fever. Occasionally, an adult worm migrates to the appendix, bile duct, or pancreatic duct, causing obstruction and inflammation of the organ. Heavier worm loads may produce abdominal pain and malabsorption of fat, protein, carbohydrate, and vitamins. In marginally nourished children, growth may be retarded. Occasionally a bolus of worms may form and produce intestinal obstruction, particularly in children. Worm loads of 50 are not uncommon, and as many as 2000 worms have been recovered from a single child. In the United States, where worm loads tend to be modest, obstruction occurs in 2 per 1000 infected children per year. The mortality in these cases is 3%. Estimates of deaths from ascariasis range from 8000 to 100,000 annually worldwide.

Infections asymptomatic with small worm loads

Malabsorption and occasional obstruction produced with heavy worm loads

### DIAGNOSIS

The diagnosis is generally made by finding the characteristic eggs in the feces. The extreme productivity of the female ascarid generally makes this task an easy one, except when the atypical-appearing unfertilized eggs predominate. The pulmonary phase of ascariasis is diagnosed by the finding of larvae and eosinophils in the sputum.

Stool examination readily reveals characteristic eggs

### TREATMENT AND PREVENTION

Albendazole, mebendazole and pyrantel pamoate are highly effective; the first two are preferred if *T. trichiura* is also present. Community-wide control of ascariasis can be achieved with mass therapy administered at 6-month intervals. Ultimately, control requires adequate sanitation facilities.

# *Hookworms*

## ANCYLOSTOMA AND NECATOR: PARASITOLOGY

Two species, *N. americanus* and *A. duodenale,* infect humans. Adults of both species are pinkish-white and measure about 10 mm in length (Fig 55–4). The head is often curved in a direction opposite that of the body, giving these worms the hooked appearance from which their common name is derived. The males have a unique fan-shaped copulatory bursa, rather than the curved, pointed tail common to the other intestinal nematodes. The

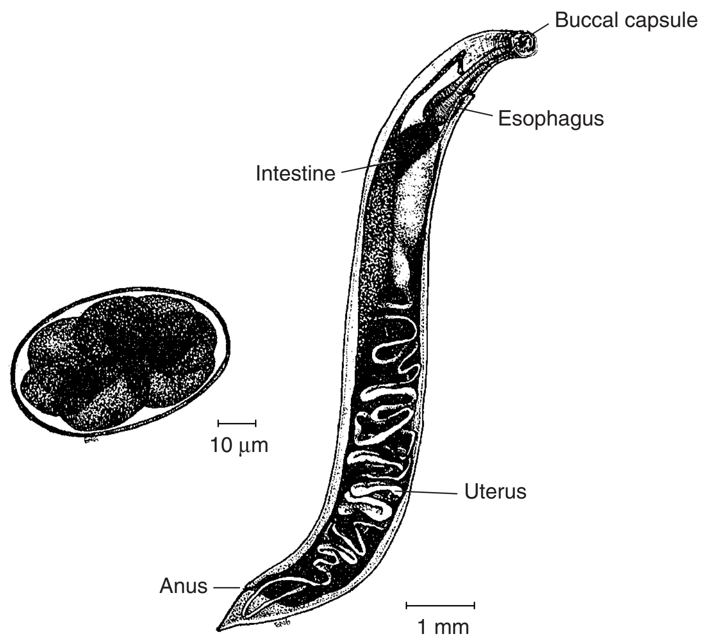



FIGURE 55–4
Female hookworm (*Necator americanus*) and egg.

*N. americanus* and *A. duodenale* infect humans

Species differentiated by morphology of oral cavity

two species can be readily differentiated by the morphology of their oral cavity. *A. duodenale,* the Old World hookworm, possesses four sharp toothlike structures, whereas *N. americanus,* the New World hookworm, has dorsal and ventral cutting plates. With the aid of these structures, the hookworms attach to the mucosa of the small bowel and suck blood. The fertilized female releases 10,000 to 20,000 eggs daily. They measure 40 by 60 $\mu$m, possess a thin shell, and are usually in the two- to four-cell stage when passed in the feces (see Fig 55–4).

## LIFE CYCLE

In soil, eggs mature and release rhabditiform larvae that molt to produce infective filariform larvae

Filariform larvae penetrate skin and then follow same path as *Ascaris* larvae to gut

For all practical purposes, the life cycles of the two hookworms, *N. americanus* and *A. duodenale,* are identical. The eggs are passed in the feces at the 4- to 8-cell stage of development and, on reaching soil, hatch within 48 hours, releasing rhabditiform larvae. These move actively through the surface layers of soil, feeding on bacteria and debris. After doubling in size, they molt to become infective filariform larvae, which may survive in moist conditions without feeding, for up to 6 weeks. On contact with human skin, they penetrate the epidermis, reach the lymphohematogenous system, and are passively transported to the right side of the heart and onward to the lungs. Here they rupture into alveolar spaces and, like juvenile ascarids, are coughed up, swallowed, and pass into the small intestine, where they mature to adulthood. Larvae of *A. duodenale,* if swallowed, can survive passage through the stomach and develop into adult worms in the small intestine.

# HOOKWORM DISEASE

## EPIDEMIOLOGY

Hookworm infection is found worldwide between the latitudes of 45°N and 30°S. Transmission requires deposition of egg-containing feces on shady, well-drained soil; development of larvae under conditions of abundant rainfall and high temperatures (23 to 33°C); and direct contact of unprotected human skin with resulting filariform larvae. Infections become particularly intense in closed, densely populated communities, such as tea and coffee plantations. *N. americanus* is found in the tropical areas of South Asia, Africa, and America, as well as the southern United States, where it was introduced with the African slave trade. *A. duodenale* is seen in the Mediterranean basin, the Middle East, northern India, China, and Japan. It has been estimated that together these two worms extract over 7 million L of blood each day from 700 million individuals scattered around the globe, including 700,000 in the United States, leading to 50,000 to 60,000 deaths annually.

Larvae require hot, moist conditions

Limited to tropical areas and southern United States

## PATHOGENESIS AND IMMUNITY

Each adult *A. duodenale* extracts 0.2 mL of blood daily and *N. americanus* 0.03 mL of blood. Additional blood loss may be related to the tendency of the worms to migrate within the intestine, leaving bleeding points at old sites of attachment. Because the adults may survive 2 to 14 years, the accumulated blood loss may be enormous. The infection elicits both a humoral antibody response and immediate hypersensitivity reaction in the host, but evidence that these moderate the infection is lacking. The peripheral and gut eosinophilia characteristic of this disease may play a role in the destruction of worms and/or modulation of the immediate hypersensitivity reaction.

Adult worms live in gut for years

Blood loss significant

Produce peripheral and gut eosinophilia

# HOOKWORM DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

In the overwhelming majority of infected patients, the worm burden is small and the infection asymptomatic. Clinical manifestations, when they do occur, may be related to the original penetration of the skin by the filariform larva, the migration of the larva through the lung, and/or the presence of the adult worm in the gut. Skin penetration may produce a pruritic erythematous rash and swelling, popularly known as ground itch. This manifestation is more common in infection with *N. americanus,* generally occurs between the toes, and may persist for several days. It is probably the result of prior sensitization to larval antigens.

Most infections asymptomatic depending on worm load

Pruritus at site of skin penetration

Pulmonary manifestations may mimic those seen in ascariasis, but are generally less frequent and less severe. In the gut, the adult worm may produce epigastric pain and abnormal peristalsis. The major manifestations, however—anemia and hypoalbuminemia—are the result of chronic blood loss. The severity of the anemia depends on the worm burden and intake of dietary iron. If iron intake exceeds iron loss resulting from hookworm infection, a normal hematocrit will be maintained. Commonly, however, dietary iron is ingested in a form that is poorly absorbed. As a result, severe anemia may develop over a period of months or years. In children, this condition may often precipitate heart failure or kwashiorkor. Mental, sexual, and physical development may be retarded.

Iron deficiency anemia caused by blood loss from intestinal worms

## DIAGNOSIS

The diagnosis is made by examining direct or concentrated stool for the distinctive eggs. As they are nearly identical in the two species, precise identification of the causative worm is generally not attempted. Quantitative egg counts can permit accurate estimation of worm load. If the stool is allowed to stand too long before it is examined, the eggs may hatch, releasing rhabditiform larvae. These larvae closely resemble those of *S. stercoralis* and must be differentiated from them.

Eggs of both species look the same

## TREATMENT AND PREVENTION

The anemia must be corrected. When it is mild or moderate, iron replacement is adequate. More severe anemia may require blood transfusions. The three most widely used anthelmintic agents, pyrantel pamoate, mebendazole and albendazole, are all highly effective. Prevention requires improved sanitation.

# Strongyloides

## *Strongyloides stercoralis:* PARASITOLOGY

Larvae differ slightly from hookworm

*S. stercoralis* adults measure only 2 mm in length, making them the smallest of the intestinal nematodes. The male is seldom seen within the human host, leading some authorities to believe that the female can conceive parthenogenetically in this environment. Be that as it may, the gravid female penetrates the mucosa of the duodenum, where she deposits her eggs. In severe infections, the biliary and pancreatic ducts, the entire small bowel, and the colon may be involved. The eggs hatch quickly, releasing rhabditiform larvae that reenter the bowel lumen and are subsequently passed into the stool. These larvae, which measure about 16 by 200 μm, can be distinguished from the similar larval stage of the hookworms by their short buccal cavity and large genital primordium (Figs 55–5 and 55–6).

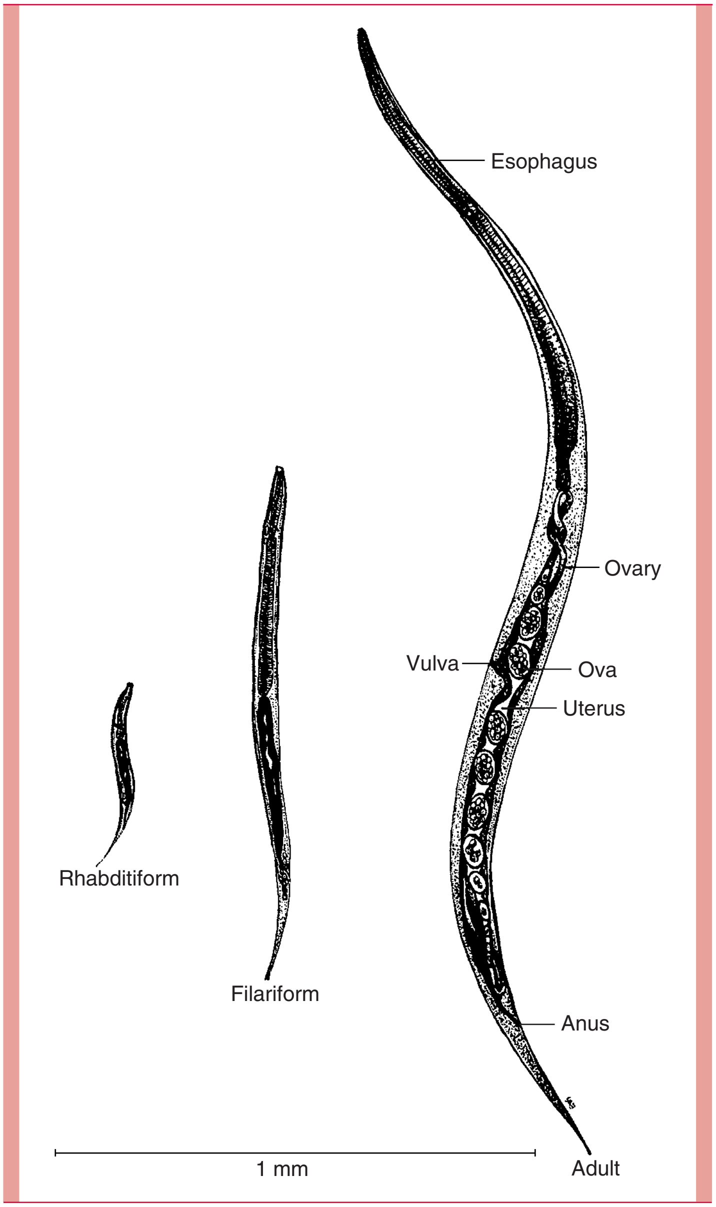



FIGURE 55–5
*Strongyloides stercoralis* worm and rhabditiform and filariform larvae.

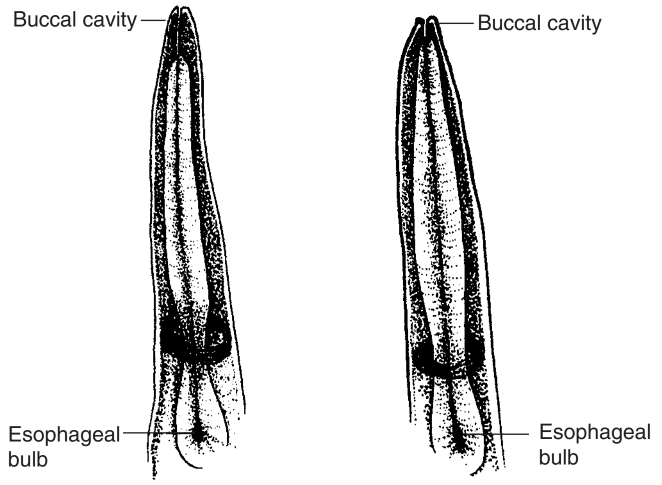



FIGURE 55–6
Anterior ends of hookworm and *Strongyloides stercoralis* rhabditiform larvae.

## LIFE CYCLE

Three different life cycles have been described for this nematode. The first, or direct cycle, is similar to that observed with the hookworms. After rhabditiform larvae are passed in the stool, they molt on soil to become filariform larvae. Filariform larvae can penetrate human skin. After transport to the lung in the vascular system, they are coughed up, swallowed and then mature to adults in the small bowel. In the second, or autoinfective cycle, the rhabditiform larva's passage through the colon to the outside world is delayed by constipation or other factors, allowing it to transform into an infective filariform larva while still within the body of its host. This larva may then invade the internal mucosa (internal autoinfection) or perianal skin (external autoinfection) without an intervening soil phase. Thus, *S. stercoralis,* unlike any of the other intestinal nematodes, has the capacity to multiply within the body of the host. The worm burden may increase dramatically, and the infection persist indefinitely, without the need for reinfection from the environment, often with dire consequence to the host. In the third, or free-living cycle, the rhabditiform larvae, after passage in the stool and deposition on the soil, develop into free-living adult males and females. These adults may propagate through several generations of free-living worms before infective filariform larvae are again produced. This cycle creates a soil reservoir that may persist even without continued deposition of feces.

Primary cycle resembles hookworm except rhabditiform larvae develop in gut

Development of filariform stage in gut produces autoinfection

Adults can develop in soil, producing sustained life cycle

# STRONGYLOIDIASIS

## EPIDEMIOLOGY

The distribution of *S. stercoralis* parallels that of the hookworms, although it is less prevalent in all but tropical areas. It infects 90 million individuals worldwide, including 400,000 throughout the rural areas of Puerto Rico and the southeastern sections of the continental United States. Although, like hookworm infection, it is generally acquired by direct contact of skin with soil-dwelling larvae, infection may also follow ingestion of filariform-contaminated food. Transformation of the rhabditiform larvae to the filariform stage within the gut can result in seeding of the perianal area with infectious organisms. These larvae may be passed to another person through direct physical contact or autoinfect the original host. In debilitated and immunosuppressed patients, transformation to the filariform stage occurs within the gut itself, producing marked autoinfection or hyperinfection.

Distribution similar to hookworm but less common

Infection by ingestion of filariform larvae also occurs

## PATHOGENESIS AND IMMUNITY

Damage to intestinal mucosa may cause malabsorptive syndrome

Immunosuppression enhances risk of autoinfection by accelerating larval development

Invasion of the intestinal epithelium may accelerate epithelial cell turnover, alter intestinal motility, and induce acute and chronic inflammatory lesions, ulcerations, and abscess formation, all of which may play a role in the malabsorptive syndrome that frequently characterizes clinical disease. Steroid- or malnutrition-related immunosuppression appears to accelerate the metamorphosis of rhabditiform to filariform larvae within the bowel lumen, enhancing the frequency and intensity of autoinfection. There is little evidence that protective immunity develops in the infected host.

# STRONGYLOIDIASIS: CLINICAL ASPECTS

## MANIFESTATIONS

Pulmonary and intestinal manifestations can be similar to hookworm, ascaris infections

Patients with strongyloidiasis do not generally give a history of "ground itch." They do, however, manifest the pulmonary disease seen in both ascariasis and, less often, in hookworm infection. The intestinal infection itself is usually asymptomatic. With heavy worm loads, however, the patient may complain of epigastric pain and tenderness, often aggravated by intake of food. In fact, peptic ulcer-like pain associated with peripheral eosinophilia strongly suggests the diagnosis of strongyloidiasis. With widespread involvement of the intestinal mucosa, vomiting, diarrhea, paralytic ileus, and malabsorption may be seen.

External autoinfection causes lesions over buttocks and back

External autoinfection produces transient, raised, red, serpiginous lesions over the buttocks and lower back that reflect larval invasion of the perianal area. If the patient is not treated, these lesions may recur at irregular intervals over a period of decades; they are particularly common after recovery from a febrile illness. Over 25% of British and American servicemen imprisoned in Southeast Asia during World War II continued to demonstrate such lesions prior to diagnosis and treatment some 40 years after exposure.

Massive hyperinfection occurs in immunosuppressed but uncommon in AIDS

Massive hyperinfection may occur in immunosuppressed patients, especially in those receiving glucocorticoid therapy, producing severe enterocolitis and widespread dissemination of the larvae to extraintestinal organs, including the heart, lungs, and central nervous system. Inexplicably, this phenomenon has been unusual in acquired immunodeficiency syndrome (AIDS) patients, even in areas where strongyloidiasis is highly endemic. The larvae may carry enteric bacteria with them, producing Gram-negative bacteremia and occasionally Gram-negative meningitis that may result in death.

## DIAGNOSIS

Rhabditiform larvae detected in stool or duodenal aspirates

The diagnosis is usually made by finding the rhabditiform larvae in the stool. Preferably, only fresh specimens should be examined to avoid the confusion induced by the hatching of hookworm eggs with the release of their look-alike larvae. The number of larvae passed in the stool varies from day to day, often requiring the examination of several specimens before the diagnosis of strongyloidiasis can be made. When absent from the stool, larvae may sometimes be found in duodenal aspirates or jejunal biopsy specimens. If the pulmonary system is involved, the sputum should be examined for the presence of larvae. Agar plate culture methods may recover organisms that go undetected by microscopic examination. Enzyme-linked immunosorbent assays for antibodies to excretory–secretory or somatic antigens are now available in reference laboratories.

## TREATMENT AND PREVENTION

Treatment essential to prevent autoinfection cycle

All infected patients should be treated to prevent the buildup of the worm burden by autoinfection and the serious consequences of hyperinfection. The drugs of choice are ivermectin and thiabendazole. In hyperinfection syndromes, therapy must be extended for 1 week. The cure rate is significantly less than 100%, and stools should be checked after therapy to see if retreatment is indicated. Patients who have resided in an endemic area at some time in their lives should be examined for the presence of this parasite both before and during steroid treatment or immunosuppressive therapy. Medical personnel caring for patients with hyperinfection syndromes should wear gowns and gloves, because stool, saliva, vomitus, and body fluids may contain infectious filariform larvae.

Medical personnel can be infected with filariform larvae

## ADDITIONAL READING

Chan MS. The global burden of intestinal nematode infections — fifty years on. *Parasitol Today* 1997;13:438. A much needed update to a classic survey of nematode infections worldwide.

Genta RM. Dysregulation of *Strongyloidiasis:* A new hypothesis. *Clin Microbiol Rev* 1992;5:345–355. A thorough, provocative, and iconoclastic review of the factors responsible for disseminated strongyloidiasis.

Prociv P. Immune responses in hookworm infections. *Clin Microbiol Rev* 2001;14:689–703.

Russell LJ. The pinworm, *Enterobius vermicularis. Prim Care* 1991;18:13–24. A comprehensive review of this common infection.

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CHAPTER 56

# Tissue Nematodes

JAMES J. PLORDE

The nematodes discussed in this chapter induce disease through their presence in the tissues and lymphohematogenous system of the human body. They are a heterogeneous group. Three of them, *Toxocara canis, Trichinella spiralis,* and *Ancylostoma braziliense,* are natural parasites of domestic and wild carnivores. Although capable of infecting humans, they cannot complete their life cycle in this host. Humans therefore serve only as injured bystanders, rather than major participants, in the life cycle of these parasites (Table 56–1).

The remaining four major nematodes, *Wuchereria bancrofti, Brugia malayi, Onchocerca volvulus,* and *Loa loa,* are members of a single superfamily (Filarioidea), and all use humans as their natural definitive host (see Table 56–1). The thin, thread-like adults live for years in the subcutaneous tissues and lymphatic vessels, where they discharge their live-born offspring or microfilariae. These progeny circulate in the blood or migrate in the subcutaneous tissues until they are ingested by a specific bloodsucking insect. Within this vector, they transform into filariform larvae capable of infecting another human when the invertebrate host again takes a blood meal.

The nematodes considered, diseases caused, and usual routes of infection in humans are listed in Table 56–1.

## TOXOCARA

### *Toxocara canis:* PARASITOLOGY

*T. canis* is a large, intestinal ascarid of canines, including dogs, foxes, and wolves. Each female worm discharges approximately 200,000 thick-shelled eggs daily into the fecal stream. After reaching the soil, these eggs embryonate for a minimum of 2 to 3 weeks. Thereafter, the eggs are infectious to both canines and humans and, in moist soil, may remain so for months to years. When ingested by a young dog, the larvae exit from the eggshell, penetrate the intestinal mucosa, and migrate through the liver and the right side of the heart to the lung. Here, like the offspring of *Ascaris lumbricoides,* they burst into the alveolar airspaces and are coughed up and swallowed; thereafter, they mature in the small bowel. In fully grown dogs, most of the migrating larvae pass through the pulmonary capillaries and reach the systemic circulation.

Cycle in canines resembles ascariasis in humans

Eggs embryonate 2–3 weeks in soil

TABLE 56–1

**General Characteristics of Tissue Nematodes**

| Parasite | Disease | Usual Source of Human Infection |
|---|---|---|
| *Toxocara canis* | Toxocariasis (visceral larva migrans) | Ingestion of ova from canine stools |
| *Trichinella spiralis* | Trichinosis | Ingestion of improperly cooked pork |
| *Ancylostoma braziliense* | Cutaneous larva migrans | Soil contaminated with dog or cat feces |
| Major filarial worms | | |
| *Wuchereria bancrofti, Brugia malayi* | Lymphatic filariasis (elephantiasis) | Mosquito |
| *Onchocerca volvulus* | Onchocerciasis (river blindness) | *Simulium* flies |
| *Loa loa* (eye worm) | Loiasis (Calabar swellings) | Deer flies |

Transplacentally infected puppies and infected lactating bitches excrete numerous ova

These larvae eventually are filtered out and encyst in the tissues. Hormonal changes and/or diminished immunity in the pregnant bitch stimulate the larvae to resume development, migrate across the placenta, and infect the unborn pups. Larvae may also pass to the newborn puppies in their mother's milk. Approximately 4 weeks after parturition, both the puppies and the lactating mother begin to pass large numbers of eggs in their stools. The mother may be superinfected by ingesting the newly passed eggs and can redevelop clinical symptoms.

Transmission to humans by ingestion of ova, and larvae invade tissues

When humans ingest infectious eggs, the liberated larvae are small enough to pass through the pulmonary capillaries and reach the systemic circulation. Rarely does the organism break into the alveoli and reach the intestine to complete its maturation to adulthood. Larvae in the systemic circulation continue to grow. When their size exceeds the diameter of the vessel through which they are passing, they penetrate its wall and enter the tissue. The larvae induce a $T_H2$-type CD4+ response characterized by eosinophilia and IgE production.

# TOXOCARIASIS

## EPIDEMIOLOGY

Soil extensively contaminated with ova deposited by domestic animals

Children are most often infected

Infection much more common than disease, but disease underreported

*T. canis* is a cosmopolitan parasite. The infection rate in the 50 million dogs inhabiting the United States is very high; over 80% of puppies and 20% of older animals are involved. "Man's best friend" deposits more than 3500 tons of feces daily in the streets, yards, and parks of America, and there is a real health risk. In some areas, between 10 and 30% of soil samples taken from public parks have contained viable *Toxocara* eggs. Moreover, serologic surveys of humans indicate that approximately 4 to 20% of the population has ingested these eggs at some time. The incidence of infection appears to be higher in the southeastern sections of the United States; presumably the warm, humid climate prolongs survival of the eggs, thereby increasing exposure. Indeed, seroprevalence rates of more than 50% have been noted in some developing nations. The presence of puppies in the home increases the risk of infection. Clinical manifestations occur predominantly among children 1 to 6 years of age; many have a history of geophagia, suggesting that disease transmission results from direct ingestion of eggs in the soil. Most infections are subclinical, but the incidence of overt disease, although difficult to assess, is certainly underreported. Serious ocular infection by larvae is frequently seen by ophthalmologists.

## TOXOCARIASIS: CLINICAL ASPECTS

### MANIFESTATIONS

Larvae that reach the systemic circulation may invade any tissue of the body, where they can induce necrosis, bleeding, and the formation of eosinophilic granulomas and, subsequently, fibrosis. The liver, lungs, heart, skeletal muscle, brain, and eye are involved most frequently. The severity of clinical manifestations is related to the number and location of these lesions and the degree to which the host has become sensitized to larval antigens. Children with more intense infection may have fever and an enlarged, tender liver. Those who are seriously ill may develop a skin rash, an enlarged spleen, asthma, recurrent pulmonary infiltrates and abdominal pain, sleep and behavioral changes, focal neurologic defects, and convulsions. Illness often persists for weeks to months, a condition frequently referred to as visceral larva migrans. Death may result from respiratory failure, cardiac arrhythmia, or brain damage. In older children and adults, systemic manifestations are uncommon. Eye invasion by larvae (ocular larva migrans) is more common. Typically, unilateral strabismus (squint) or decreased visual acuity causes the patient to consult an ophthalmologist. Examination reveals granulomatous endophthalmitis, which is usually a reaction to a larva that is already dead; it is sometimes mistaken for malignant retinoblastoma, and an unnecessary enucleation is performed.

Any tissue invaded by larvae

Disease results from organ invasion and hypersensitivity

Ocular invasion produces granulomatous endophthalmitis

### DIAGNOSIS

Stool examination is not helpful, because the parasite seldom reaches adulthood in humans. Definitive diagnosis requires demonstration of the larva in a liver biopsy specimen or at autopsy. A presumptive diagnosis may be made based on the clinical picture; eosinophilic leukocytosis; elevated levels of IgE; and on elevated antibody titers to blood group antigens, particularly the group A antigen. An enzyme immunoassay (EIA) using larval antigens has been developed, providing clinicians with a reasonably sensitive (75%) and specific (90%) serologic test. A Western blot procedure is somewhat more sensitive but is not widely available. Unfortunately, many patients with related ocular infections remain seronegative; some demonstrate elevated aqueous humor titers.

Tissue biopsy required for detection

Serodiagnosis using EIA reliable

### TREATMENT AND PREVENTION

Corticosteroid treatment may be lifesaving if the patient has serious pulmonary, myocardial, or central nervous system involvement. Anthelmintic therapy with albendazole or mebendazole is generally administered, although the efficacy of these drugs remains uncertain. Prevention requires control of indiscriminate defecation by dogs and repeated worming of household pets. Worming must begin when the animal is 3 weeks of age and be repeated every 3 months during the first year of life and twice a year thereafter.

Corticosteroids helpful in serious disease

Worming of household pets important

# *TRICHINELLA*

## *Trichinella spiralis*: PARASITOLOGY

Adult *Trichinella* live in the duodenal and jejunal mucosa of flesh-eating animals throughout the world, particularly swine, rodents, bears, canines, felines, and marine mammals. Originally thought to be members of a single species, arctic, temperate, and tropical strains of *Trichinella* demonstrate significant epidemiologic and biologic differences and have

Intestinal parasite of many flesh-eating mammals recently been reclassified into seven distinct species. Only two species, *T. spiralis* and the arctic species *T. nativa,* display a high level of pathogenicity for humans. This discussion focuses on the former, while highlighting the unique epidemiologic and clinical characteristics of the latter.

The tiny (1.5-mm) male copulates with his outsized (3.5-mm) mate and, apparently spent by the effort, dies. Within 1 week, the inseminated female begins to discharge offspring. Unlike those of most nematodes, these progeny undergo intrauterine embryonation and are released as second-stage larvae. The birthing continues for the next 4 to 16 weeks, resulting in the generation of some 1500 larvae, each measuring 6 by 100 $\mu$m.

Larvae reach striated muscle and encapsulate but are still viable

Eating infected flesh spreads the disease

From their submucosal position, the larvae find their way into the vascular system and pass from the right side of the heart through the pulmonary capillary bed to the systemic circulation, where they are distributed throughout the body. Larvae penetrating tissue other than skeletal muscle disintegrate and die. Those finding their way to striated muscle continue to grow, molt, and gradually encapsulate over a period of several weeks. Calcification of the cyst wall begins 6 to 18 months later, but the contained larvae may remain viable for 5 to 10 years. The muscles invaded most frequently include the extraocular muscles of the eye, the tongue, the deltoid, pectoral, and intercostal muscles, the diaphragm, and the gastrocnemius. If a second animal feeds on the infected flesh of the original host, the encysted larvae are freed by gastric digestion, penetrate the columnar epithelium of the intestine, and mature just above the lamina propria.

# TRICHINOSIS

## EPIDEMIOLOGY

Swine infected by eating rats or meat in garbage

Human infection most often from undercooked pork

Wild animals (eg, bear, walrus) also a risk

Trichinosis is widespread in carnivores. Among domestic animals, swine are most frequently involved. They acquire the infection by eating rats or garbage containing cyst-laden scraps of uncooked meat. Human infection, in turn, results largely from the consumption of improperly prepared pork products. In the United States, most outbreaks have been traced to ready-to-eat pork sausage prepared in the home or in small, unlicensed butcheries. Disease incidence is highest in Americans of Polish, German, and Italian descent, presumably because of their custom of producing and eating such sausage during holidays. Recent outbreaks have been reported among Indochinese refugees, apparently related to undercooking of fresh pork. Clusters have also followed feasts of wild pig in California and Hawaii. At present, nearly one third of human cases in the United States, particularly those in Alaska and other western states, have been attributed to consumption of the meat of wild animals, particularly bears. Outbreaks among Alaskan and Canadian Inuit populations have followed the ingestion of raw *T. nativa*–infected walrus meat. Several recent outbreaks in Europe have involved horse meat or wild boar. Each year, a few cases are acquired from ground beef intentionally but illegally adulterated with pork.

Prevalence declining due to cooking and freezing of pork

Human infections usually are subclinical

Human infections occur worldwide. In the United States, the prevalence of cysts found in the diaphragms of patients at autopsy has declined from 16.1 to 4.2% over a period of 30 years. This decline has been attributed to decreased consumption of pork and pork products; federal guidelines for the commercial preparation of such foodstuffs; the widespread practice of freezing pork, which kills all but arctic strains of *Trichinella*; and legislation requiring the thorough cooking of any meat scraps to be used as hog feed. Nevertheless, it is estimated that more than 1.5 million Americans carry live *Trichinella* in their musculature and that 150,000 to 300,000 acquire new infection annually. Fortunately, the overwhelming majority are asymptomatic, and only about 100 clinically recognized cases are reported annually to federal officials. In other areas of the world, infection is more commonly acquired from sylvatic sources, including wild boar, bush pigs, and warthogs.

## PATHOGENESIS AND IMMUNITY

The pathologic lesions of trichinosis are related almost exclusively to the presence of larvae in the striated muscle, heart, and central nervous system. Invaded muscle cells enlarge, lose their cross-striations, and undergo a basophilic degeneration. Surrounding the involved area is an intense inflammatory reaction consisting of neutrophils, lymphocytes, and eosinophils. With the development of specific IgG and IgM antibodies, eosinophil-mediated destruction of circulating larvae begins, production of new larvae is slowed, and the expulsion of adult worms is hastened. A vasculitis demonstrated in some patients has been attributed to deposition of circulating immune complexes in the walls of the vessels.

Larvae in striated muscle, heart, and central nervous system

Acute inflammatory reaction with eosinophil-mediated destruction of larvae

# TRICHINOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

One or two days after the host has ingested tainted meat, the newly matured adults penetrate the intestinal mucosa, producing nausea, abdominal pain, and diarrhea. In mild infections, these symptoms may be overlooked, except in a careful retrospective analysis; in more serious infections, they may persist for several days and render the patient prostrate. Diarrhea persisting for a period of weeks has been characteristic of *T. nativa* outbreaks following ingestion of walrus meat by the Inuit population of northern Canada. Larval invasion of striated muscle begins approximately 1 week later and initiates the longer (6 weeks) and more characteristic phase of the disease. Patients in whom 10 or fewer larvae are deposited per gram of tissue are usually asymptomatic; those with 100 or more generally develop significant disease; and those with 1000 to 5000 have a very stormy course that occasionally ends in death. Fever, muscle pain, muscle tenderness, and weakness are the most prominent manifestations. Patients may also display eyelid swelling, a maculopapular skin rash, and small hemorrhages beneath the conjunctiva of the eye and the nails of the digits. Hemoptysis and pulmonary consolidation are common in severe infections. If there is myocardial involvement, electrocardiographic abnormalities, tachycardia, or congestive heart failure may be seen. Central nervous system invasion is marked by encephalitis, meningitis, and polyneuritis. Delirium, psychosis, paresis, and coma can follow.

Initial abdominal pain and diarrhea as adults penetrate

Symptoms depend on number and extent of larval muscle invasion

Severe complications include hemoptysis and heart failure

## DIAGNOSIS

The most consistent abnormality is an eosinophilic leukocytosis during the second week of illness and persists for the remainder of the clinical course. Eosinophils typically range from 15 to 50% of the white cell count, and in some patients, this may induce extensive damage to the cardiac endothelium. In severe or terminal cases, the eosinophilia may disappear altogether. Serum levels of IgE and muscle enzymes are elevated in most clinically ill patients.

Eosinophilia up to 50% from second week on

There are a number of valuable serologic tests, including indirect fluorescent antibody, bentonite flocculation, and enzyme-linked immunosorbent assay. Significant antibody titers are generally absent before the third week of illness, but may then persist for years.

Antibody usually appears after 2 weeks and then persists

Biopsy of the deltoid or gastrocnemius muscles during the third week of illness often reveals encysted larvae.

Muscle biopsy reveals larvae

## TREATMENT

Patients with severe edema, pulmonary manifestations, myocardial involvement, or central nervous system disease are treated with corticosteroids. The value of specific anthelmintic therapy remains controversial. The mortality of symptomatic patients is 1%, rising to 10% if the central nervous system is involved. Mebendazole and albendazole halt the production of new larvae, but in severe infection, the destruction of tissue larvae may provoke a hazardous hypersensitivity response in the host. This may be moderated with corticosteroids.

Corticosteroids used in severe cases

## PREVENTION

Primary prevention involves thorough cooking

Control of trichinosis requires adherence to federal feeding regulations for pigs, and limiting contact between domestic pigs and wild animals, particularly rodents, who might be carry trichinella larvae in their tissues. Domestically, care should be taken to cook pork to an internal temperature of at least 76.6°C, freeze it at –15°C for 3 weeks, or thoroughly smoke it before it is ingested. *T. nativa* in the flesh of arctic animals may survive freezing for a year or more. All strains may survive apparently adequate cooking in microwave ovens due to the variability in the internal temperatures achieved.

# Cutaneous Larva Migrans

Caused usually by larvae of dog and cat hookworms

Filariform larvae penetrate and migrate in human skin

Cutaneous larva migrans, or creeping eruption, is an infection of the skin caused by the larvae of a number of animal and human parasites, most commonly the dog and cat hookworm *Ancylostoma braziliense.* Eggs discharged in the feces of infected animals and deposited on warm, moist, sandy soil develop filariform larvae capable of penetrating mammalian skin on contact. In the United States, parasite transmission is particularly common in the beach areas of the southern Atlantic and Gulf states.

Adult forms do not develop in humans

Although larvae do not develop further within humans, they may migrate within the skin for a period of weeks to months. Clinically, the patient notes a pruritic, raised, red, irregularly linear lesion 10 to 20 cm long. Skin excoriation from scratching enhances the likelihood of secondary bacterial infection. Half of infected patients develop Löffler's syndrome of transient, migratory pulmonary infiltrations associated with peripheral eosinophilia. The syndrome most probably reflects pulmonary migration of larvae. Larvae are rarely found in either sputum or skin biopsies, and the diagnosis must be established on clinical grounds.

The disease responds well to albendazole, ivermectin, or topical thiabendazole. Antihistamines and antibiotics may be helpful in controlling pruritus and secondary bacterial infection, respectively.

# Lymphatic Filaria

Lymphatic filariasis encompasses a group of diseases produced by certain members of the superfamily Filarioidea that inhabit the lymphatic system of humans. Their presence induces an acute inflammatory reaction, chronic lymphatic blockade, and in some cases, grotesque swellings of the extremities and genitalia known as elephantiasis.

## WUCHERERIA AND BRUGIA: PARASITOLOGY

Adult worms live in lymphatic vessels for a decade

Microfilariae develop from ova

The two agents most commonly responsible for lymphatic filariasis are *W. bancrofti* and *B. malayi.* Both are thread-like worms that lie coiled in the lymphatic vessels, male and female together, for the duration of their decade-long lifespan. The female *W. bancrofti* measures 100 mm in length, and the male 40 mm. *B. malayi* adults are approximately half these sizes. The gravid females produce large numbers of embryonated eggs. At oviposition, the embryos uncoil to their full length (200 to 300 $\mu$m) to become microfilariae. The shell of the egg elongates to accommodate the embryo and is retained as a thin, flexible sheath. Although the offspring of the two species resemble each other, they may be differentiated on

TABLE 56–2

**Differentiation of Microfilariae**

| Parasite | Location | Sheath | Size (μm) | Nuclei of Tail | Periodicity |
|---|---|---|---|---|---|
| *Wuchereria bancrofti* | Blood | Yes | 360 | None | Usually nocturnal |
| *Brugia malayi* | Blood | Yes | 220 | Two | Nocturnal |
| *Loa loa* | Blood | Yes | 275 | Continuous | Diurnal |
| *Onchocerca volvulus* | Skin | No | 300 | None | None |

the basis of length, staining characteristics, and internal structure (Table 56–2). The microfilariae eventually reach the blood. In most *W. bancrofti* and *B. malayi* infections, they accumulate in the pulmonary vessels during the day. At night, in response to changes in oxygen tension, they spill out into the peripheral circulation, where they are found in greatest numbers between 9 PM and 2 AM. A Polynesian strain of *W. bancrofti* displays a different periodicity, with the peak concentration of organisms occurring in the early evening. Periodicity has an important epidemiologic consequence, because it determines the species of mosquito to serve as vector and intermediate host. Within the thoracic muscles of the mosquito, microfilariae are transformed first into rhabditiform and then into filariform larvae. The latter actively penetrate the feeding site when the mosquito takes its next meal. Within the new host, the parasite migrates to the lymphatic vessels, undergoes a series of molts, and reaches adulthood in 6 to 12 months.

Microfilariae circulate in peripheral blood once each day

Mosquito is essential vector and intermediate host

## LYMPHATIC FILARIASIS

### EPIDEMIOLOGY

Lymphatic filariasis currently infects about 120 million individuals in Africa, Latin America, the Pacific Islands, and Asia; more than 75% of these cases are concentrated in Asia. *W. bancrofti,* transmitted primarily by mosquitoes of the genera *Anopheles* or *Culex,* is the more cosmopolitan of the two species; it is found in patchy distribution throughout the poorly sanitated, densely crowded urban areas of all three continents. A small endemic focus once existed near Charleston, South Carolina, but died out in the 1920s. Moreover, some 15,000 *W. bancrofti* infections were acquired by American servicemen during World War II. The same infection has recently been found in approximately 7% of Haitian refugees to the United States.

Primarily in Asia and other tropical areas

*B. malayi,* transmitted by mosquitoes of the genus *Mansonia,* is confined to the rural coastal areas of Asia and the South Pacific. Strains with an unusual periodicity have been found in animals. Humans are the only known vertebrate hosts for most strains of *B. malayi* and for *W. bancrofti.* In the eastern Indonesian archipelago, a closely related species, *B. timori,* is transmitted by night-feeding anopheline mosquitoes.

Humans are the only vertebrate hosts for *Wuchereria*

### PATHOLOGY AND PATHOGENESIS

Pathologic changes, which are confined primarily to the lymphatic system, can be divided into acute and chronic lesions. In acute disease, the presence of molting adolescent worms and dead or dying adults stimulates dilatation of the lymphatics, hyperplastic changes in the vessel endothelium, infiltration by lymphocytes, plasma cells, and eosinophils, and thrombus formation (ie, acute lymphangitis). These developments are followed by granuloma formation, fibrosis, and permanent lymphatic obstruction. Repeated infections eventually result in massive lymphatic blockade. The skin and subcutaneous tissues become edematous, thickened, and fibrotic. Dilated vessels may rupture, spilling lymph into the tissues or body cavities. Bacterial and fungal superinfections of the skin often supervene and contribute to tissue damage.

Lymphatic blockade with repeated infections

# LYMPHATIC FILARIASIS: CLINICAL ASPECTS

## MANIFESTATIONS

Lymphadenitis, urticaria, and eosinophilia are early findings

Acute manifestations can recur

Elephantiasis may be end result

For tropical eosinophilia syndrome, microfilaria not found in blood

Individuals who enter endemic areas as adults and reside therein for months to years often present with acute lymphadenitis, urticaria, eosinophilia, and elevated serum IgE levels; they seldom go on to develop lymphatic obstruction. A significant proportion of indigenous populations present with asymptomatic microfilaremia. Some of these spontaneously clear their infection, and others go on to experience "filarial fevers" and lymphadenitis 8 to 12 months after exposure. The fever is typically low grade; in more serious cases, however, temperatures as high as 40°C, chills, muscle pains, and other systemic manifestations may be seen. Classically, the lymphadenitis is first noted in the femoral area as an enlarged, red, tender lump. The inflammation spreads centrifugally down the lymphatic channels of the leg. The vessels become enlarged and tender, the overlying skin red and edematous. In Bancroftian filariasis, the lymphatic vessels of the testicle, epididymis, and spermatic cord are frequently involved, producing a painful orchitis, epididymitis, and funiculitis; inflamed retroperitoneal vessels may simulate acute abdomen. Epitrochlear, axillary, and other lymphatic vessels are involved less frequently. The acute manifestations last a few days and resolve spontaneously, only to recur periodically over a period of weeks to months. With repeated infection, permanent lymphatic obstruction develops in the involved areas. Edema, ascites, pleural effusion, hydrocele, and joint effusion result. The lymphadenopathy persists and the palpably swollen lymphatic channels may rupture, producing an abscess or draining sinus. Rupture of intra-abdominal vessels may give rise to chylous ascites or urine. In patients heavily and repeatedly infected over a period of decades, elephantiasis may develop. Such patients may continue to experience acute inflammatory episodes.

In southern India, Pakistan, Sri Lanka, Indonesia, Southeast Asia, and East Africa, an aberrant form of filariasis is seen. This form, termed tropical pulmonary eosinophilia, is characterized by an intense eosinophilia, elevated levels of IgE, high titers of filarial antibodies, the absence of microfilariae from the circulating blood, and a chronic clinical course marked by massive enlargement of the lymph nodes and spleen (children) or chronic cough, nocturnal bronchospasm, and pulmonary infiltrates (adults). Untreated, the disease may progress to pulmonary interstitial fibrosis. Microfilariae have been found in the tissues of such patients, and the clinical manifestations may be terminated with specific antifilarial treatment. It is believed that this syndrome is precipitated by the removal of circulating microfilariae by an IgG-dependent, cell-mediated immune reaction. Microfilariae are trapped in various tissue sites where they incite an eosinophilic inflammatory response, granuloma formation, and fibrosis.

## DIAGNOSIS

Eosinophilia during acute episodes

Search for microfilariae in the blood requires careful timing

Eosinophilia is usually present during the acute inflammatory episodes, but definitive diagnosis requires the presence of microfilaria in the blood or lymphatic, ascitic, or pleural fluid. They are sought in Giemsa- or Wright-stained thick and thin smears. The major distinguishing features of these and other microfilariae are listed in Table 56–2. Because the appearance of the microfilariae is usually periodic, specimen collection must be properly timed. If this procedure proves difficult, the patient may be challenged with the antifilarial agent diethylcarbamazine (DEC). This drug stimulates the migration of the microfilariae from the pulmonary to the systemic circulation and enhances the possibility of their recovery. If the parasitemia is scant, the specimen may be concentrated before it is examined. Once found, the microfilariae must be differentiated from those produced by other species of filariae. A number of serologic tests have been employed for the diagnosis of microfilaremic disease, but until recently they have lacked adequate sensitivity and specificity; even the more recent tests are of little diagnostic significance in individuals indigenous to the endemic area, because many people have experienced a prior filarial infection. Circulating filarial antigens can be found in most microfilaremic patients and also in some seropositive amicrofilaremic individuals. Antigen detection may thus prove to be a specific indicator of active disease. Tropical eosinophilia is diagnosed as described previously.

## TREATMENT

DEC eliminates the microfilariae from the blood and kills or injures the adult worms, resulting in long-term suppression of the infection or parasitologic cure. Frequently the dying microfilariae stimulate an allergic reaction in the host. This response is occasionally severe, requiring the use of antihistamines and corticosteroids. The role of ivermectin in the treatment of lymphatic filariasis has not yet been established. Early studies have demonstrated a high level of effectiveness in clearing microfilaremia following the administration of a single dose. The tissue changes of elephantiasis are often irreversible, but the enlargement of the extremities may be ameliorated with pressure bandages or plastic surgery. Control programs combine mosquito control with mass treatment of the entire population.

Killing of microfilariae may stimulate allergic response

# ONCHOCERCA

Onchocerciasis or river blindness, produced by the skin filaria *O. volvulus,* is characterized by subcutaneous nodules, thickened pruritic skin, and blindness.

## *Onchocerca volvulus:* PARASITOLOGY

The 40- to 60-cm thread-like female adults lie, together with their diminutive male partners, in coiled masses within fibrous subcutaneous and deep tissue nodules. The female gives birth to more than 2000 microfilariae each day of her 15-year lifespan. These progeny lose their sheaths soon after leaving the uterus, exit from the fibrous capsule, and migrate for up to 2 years in the subcutaneous tissues, skin, and eye. Ultimately they die or are ingested by black flies of the genus *Simulium,* which breed along the banks of turbulent, fast-moving streams. After transformation into filariform larvae, they are transmitted to another human host. There they molt repeatedly over 6 to 12 months before reaching adulthood and becoming encapsulated.

Adults in subcutaneous tissue, skin, and eye

Transmitted by *Simulium* fly

## ONCHOCERCIASIS

### EPIDEMIOLOGY

Onchocerciasis infects approximately 13 to 20 million persons, rendering 1 to 5% of them blind. The vast majority of the afflicted live in tropical Africa, over half of these in Nigeria and Congo. Foci of infection are also found in Yemen, Saudi Arabia, and Latin America from southern Mexico through the northern half of South America. It has been suggested that the disease was introduced into South America by West Africans enslaved and transported to the New World for the purpose of mining gold in the mountain streams of Venezuela and Colombia. The Central American foci date from Napoleon III's use of Sudanese troops to support his invasion of Mexico in 1862. The disease still persists on the high slopes of the Sierra, where coffee plantations lie along the rapidly flowing streams that serve as breeding places for *Simulium* species.

Most cases in tropical Africa

## ONCHOCERCIASIS: CLINICAL ASPECTS

### MANIFESTATIONS

Subcutaneous nodules may be multiple with hypersensitivity reaction to microfilariae

Important cause of blindness in affected areas

The subcutaneous nodules that harbor the adult worms can be located anywhere on the body, generally over bony prominences. In Mexico and Guatemala, where the fly vector typically bites the upper part of the body, they are concentrated on the head; in South America and Africa, they are found primarily on the trunk and legs. Although nodules may number in the hundreds, most infected individuals have less than 10. They are firm, freely movable, and measure 1 to 3 cm in diameter. Unless the nodule is located over a joint, pain and tenderness are unusual. Of greater consequence to the patient are the side effects of the presence of microfilariae in the tissues. An immediate hypersensitivity reaction to antigens released by dead or dying parasites results in acute and chronic inflammatory reaction. In the skin, this reaction is manifested as a papular or erysipelas-like rash with severe itching. In time, the skin thickens and lichenifies. As subepidermal elastic tissue is lost, wrinkles and large skin folds or hanging groins are formed. In parts of Africa, fibrosing, obstructive lymphadenitis may result in elephantiasis. Invasion of the eye, however, causes the most devastating lesions. Punctate keratitis, iritis, and chorioretinitis can lead to a decrease in visual acuity and, in time, total blindness. In Central America, eye lesions may be seen in up to 30% of infected patients. In certain communities in West Africa, 85% of the population has ocular lesions and 50% of the adult male population is blind.

### DIAGNOSIS

Microfilariae seen in skin samples

The diagnosis is made by demonstrating the microfilariae in a thin skin snips taken from an involved area. When the eye is involved, the organism may sometimes be seen in the anterior chamber with the help of a slit lamp.

### TREATMENT AND PREVENTION

Treatment may cause hypersensitivity reactions

Traditionally, DEC has been used to kill the microfilariae. Treatment was begun with very small doses to prevent rapid parasite destruction and the attendant allergic consequences. This consideration was particularly important when the eye was involved; a treatment-induced inflammatory reaction can damage it further. The newer microfilaremic agent, ivermectin, has been demonstrated to be more effective than DEC and does not appear to induce the severe allergic manifestations seen with the latter agent. Because it does not kill the adult worm, retreatment over a period of several years is necessary. No satisfactory methods of control have yet been developed. Application of insecticides to the vector's breeding waters must be sustained for decades to disrupt transmission permanently, because the parasite is so long-lived within humans. With the introduction of ivermectin, mass treatment or chemoprophylaxis is now possible.

There are no effective vaccines or chemoprophylactic agents. Annual mass distribution of ivermectin over a period of 10 to 15 years may interrupt the transmission cycle. A World Health Organization–funded *Simulium* larva control program utilizing aerial insecticides has succeeded in interrupting transmission of onchocerciasis in the savanna regions of West Africa.

## *LOA LOA*

Loiasis is a filarial disease of West Africa produced by the eye worm, *Loa loa.* The long-lived adults migrate continuously through the subcutaneous tissues of humans at a maximum rate of about 1 cm/hr. During migration, they produce localized areas of allergic inflammation termed Calabar swellings. These egg-sized lesions persist for 2 to 3 days and may be accompanied by fever, itching, urticaria, and pain. At times, the adult worms may cross the eye subconjunctivally, producing intense tearing, pain, and alarm.

Adults migrate through subcutaneous tissues producing localized Calabar swellings

The female produces sheathed microfilariae, which are found in the bloodstream during daytime hours. Deer flies of the genus *Chrysops* serve as vectors.

The diagnosis is made by recovering the adult worm from the eye or by isolating the characteristic microfilariae from the blood or Calabar swellings. Eosinophilia is constant. DEC destroys both adults and microfilariae, but must be administered cautiously to avoid marked allergic reactions. Albendazole slowly decreases microfilarial levels without producing allergic reactions, possibly by preferential action on the adult worms.

Adult worm demonstrated in eye or microfilaria in blood or tissue

## ADDITIONAL READING

Burnham G. Onchocerciasis. *Lancet* 1998;351:1341–1346.

Jelinek T, Maiwald H, Northdurft HD, et al. Cutaneous larva migrans in travelers: synopsis of histories, symptoms, and treatment of 98 patients. *Clin Infect Dis* 1994;19:1062.

Jongwutiwes S, et al. First outbreak of human trichinellosis caused be *Trichinella pseudospiralis. Clin Infect Dis* 1998;26:111.

Klion AD, Massougbodji A, Sadeler BC, et al. Loiasis in endemic and nonendemic populations: immunologically mediated differences in clinical presentation. *J Infect Dis* 1991;163:1318–1325. Authors note the differences in clinical presentation of loiasis in visitors to endemic areas and indigenous populations, and they relate this to differences in the modulation of the immune response to parasitic antigens.

MacLean JD, Viallet J, Law C, Staudt M. Trichinosis in the Canadian Arctic: report of five outbreaks and a new clinical syndrome. *J Infect Dis* 1989;160:513–520. Trichinosis, which appears to be both common and widespread in the Arctic, is characterized by a distinct clinical presentation in which gastrointestinal manifestations predominate.

Worley G, Green JA, Frothingham TE, et al. *Toxocara canis* infection. Clinical and epidemiological associations with seropositivity in kindergarten children. *J Infect Dis* 1984;149:591–597.

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CHAPTER 57

# Cestodes

JAMES J. PLORDE

Cestodes are long, ribbon-like helminths that have gained the common appellation of tapeworm from their superficial resemblance to sewing tape. Their appearance, number, and exaggerated reputation for inducing weight loss have made them the best known of the intestinal worms. Although improvements in sanitation have dramatically reduced their prevalence in the United States, they continue to inhabit the bowels of many of its citizens. In some parts of the world, indigenous populations take purgatives monthly to rid themselves of this, the largest and most repulsive of the intestinal parasites.

## PARASITOLOGY

### MORPHOLOGY

Without gut, food absorbed from host

Divided into scolex, neck, and segmented body parts

Each proglottid a hermaphroditic unit releasing eggs via rupture or through uterine pore

Like all helminths, tapeworms lack vascular and respiratory systems. In addition, they are devoid of both gut and body cavity. Food is absorbed across a complex cuticle, and the internal organs are embedded in a solid parenchyma. The adult is divided into three distinct parts: the "head" or scolex; a generative neck; and a long, segmented body, the strobila. The scolex typically measures less than 2 mm in diameter and is equipped with four muscular sucking discs used to attach the worm to the intestinal mucosa of its host. (In one genus, *Diphyllobothrium,* the discs are replaced by two grooves, or bothria.) As a further aid in attachment, the scolex of some species possesses a retractable protuberance, or rostellum, armed with a crown of chitinous hooks. Immediately posterior to the scolex is the neck from which individual segments, or proglottids, are generated one at a time to form the chain-like body. Each proglottid is a self-contained hermaphroditic reproductive unit joined to the remainder of the colony by a common cuticle, nerve trunks, and excretory canals. Its male and female gonads mature and effect fertilization as the segment is pushed farther and farther from the neck by the formation of new proglottids. When the segment reaches gravidity, it releases its eggs by rupturing, disintegrating, or passing them through its uterine pore. The eggs of the genus *Taenia* possess a solid shell and contain a fully developed, six-hooked (hexacanth) embryo. The eggs of *Diphyllobothrium latum,* in contrast, are immature at the time of deposition and possess a covered aperture, or operculum, through which the embryo exits once fully developed.

Cestode

### LIFE CYCLE

With the exception of *Hymenolepis nana,* further development of all cestodes requires the passage of the larvae through one or more intermediate hosts. Eggs of the genus *Taenia*

TABLE 57–1

## Intestinal and Tissue Tapeworms

| Stage | *Diphyllobothrium latum* | *Taenia saginata* | *Taenia solium* | *Hymenolepis nana* | *Echinococcus granulosus* | *Echinococcus multilocularis* |
|---|---|---|---|---|---|---|
| Adult | | | | | | |
| Definitive host | Humans, cats, dogs | Humans | Humans | Humans, rodents | Dogs, wolves | Foxes |
| Location | Gut lumen[a] | Gut lumen[a] | Gut lumen[a] | Gut lumen[a] | Gut lumen | Gut lumen |
| Length (m) | 3–10 | 4–6 | 2–4 | 0.02–0.04 | 0.005 | 0.005 |
| Attachment device | Grooves | Discs | Discs, hooklets | Discs, hooklets | Discs, hooklets | Discs, hooklets |
| Mature segment | Broad | Elongated | Elongated | Broad | Elongated | Elongated |
| Egg | | | | | | |
| Maturation status | Nonembryonated | Embryonated | Embryonated | Embryonated | Embryonated | Embryonated |
| Distinguishing characteristics | Operculate | Radial striations | Radial striations | Polar filaments | Radial striations | Radial striations |
| Larval development in humans | No | No | Yes | Yes | Yes | Yes |
| Larva | | | | | | |
| Intermediate host | Copepods, fishes | Cattle | Swine, humans | Humans, rodents | Herbivores, humans | Field mice, humans |
| Location | Tissue | Tissue | Tissue[a] | Gut mucosa[a] | Tissue[a] | Tissue[a] |
| Form | Procercoid (copepod) Plerocercoid (fish) | Cysticercus | Cysticercus | Cysticercoid | Hydatid cyst | Hydatid cyst |

[a]Site of human infection.

Eggs of *Taenia* must be ingested by intermediate host

Infectious cysts of *Taenia* form in tissues of intermediate

Definitive host ingests cysts in flesh of intermediate hosts to yield adult intestinal worms

pass in the stool of their definitive host, reach the soil, and are ingested by the specific intermediate. They hatch within its gut, and the released embryos penetrate the intestinal mucosa, find their way through the lymphohematogenous system to the tissues, and encyst therein. From the germinal lining of this cyst, immature scolices or protoscolices are formed. A cyst with a single such structure is known as a cysticercus (or, in the case of *H. nana,* a cysticercoid); a cyst with multiple protoscolices is known as a coenurus. In some species of tapeworm, daughter cysts, each containing many protoscolices, are formed within the mother or hydatid cyst. The cycle for all is completed when the definitive host ingests the cyst-ridden flesh of the intermediate host. After digestion of the surrounding meat in the stomach, the cyst is freed, and the protoscolex everts to become a scolex. Following attachment to the mucosa, a new strobila is generated.

*D. latum* requires two intermediates—a copepod and a freshwater fish—to complete cycle

*D. latum,* whose eggs are immature on release, requires two intermediates to complete its larval development. The egg must reach fresh water before the operculum opens and a ciliated, free-swimming larva, or coracidium, is released. The coracidium is then ingested by the first intermediate host, a copepod, in which it is transformed into a larva (procercoid). When the copepod is, in turn, ingested by a freshwater fish, the larva penetrates the musculature of the fish to form an elongated and infectious larva, the plerocercoid. Life cycles and characteristics of important intestinal and tissue tapeworms infecting humans are summarized in Table 57–1.

## CLINICAL DISEASE

The clinical consequences of tapeworm infection in humans depend on whether the patient serves as the primary or the intermediate host. In the former case, the adult worm is confined to the lumen of the gut, and the consequences of the infection are typically minor. Taeniasis saginata and diphyllobothriasis are prime examples. In contrast, when the patient serves as the intermediate host (eg, for *E. granulosus*), larval development produces tissue invasion and frequently serious disease. The capacity of *H. nana* and *T. solium* to use humans as both primary and intermediate hosts is unique.

Clinical effects depend on whether humans are definitive hosts or intermediate hosts

# BEEF TAPEWORM

## *Taenia saginata*: PARASITOLOGY

*T. saginata* inhabits the human jejunum, where it may live for up to 25 years and grow to a maximum length of 10 m. Its 1-mm scolex lacks hooklets but possesses the four sucking discs typical of most cestodes (Fig 57–1A). The creamy white strobila consists of 1000 to 2000 individual proglottids. The terminal segments are longer (20 mm) than they are wide (5 mm) and contain a large uterus with 15 to 20 lateral branches; these characteristics are useful in differentiating them from those of the closely related pork tapeworm, *T. solium.* When fully gravid, strings of 6 to 9 terminal proglottids, each containing approximately 100,000 eggs, break free from the remainder of the strobila. These muscular segments may crawl unassisted through the anal canal or be passed intact with the stool. Proglottids reaching the soil eventually disintegrate, releasing their distinctive eggs. These eggs are 30 to 40 $\mu$m in diameter, spherical, and possess a thick, radially striated shell (Fig 57–1B). In appropriate environments, the hexacanth embryo may survive for months. If ingested by cattle or certain other herbivores, the embryo is released, penetrates the intestinal wall, and is carried by the vascular system to the striated muscles of the tongue, diaphragm, and hindquarters. Here it is transformed into a white, ovoid (5 by 10 mm) cysticercus (*Cysticercus bovis*). When present in large numbers, cysticerci impart a spotted or "measly" appearance to the flesh. Humans are infected when they ingest inadequately cooked meat containing these larval forms.

*T. saginata* inhabits human jejunum

Gravid proglottids passed in stool

Eggs ingested by herbivore intermediates

Cysticerci in bovine striated muscle

Humans infected by eating inadequately cooked infected meat

## BEEF TAPEWORM DISEASE

### EPIDEMIOLOGY

In the United States, sanitary disposal of human feces and federal inspection of meat have nearly interrupted transmission of *T. saginata.* At present, less than 1% of examined carcasses are infected. Nevertheless, bovine cysticercosis is still a significant problem in the southwestern area of the country where cattle become infected in feedlots or while pastured on land irrigated with sewage or worked by infected laborers without access to sanitary facilities. Shipment of infected carcasses can result in human infection in other areas of the United States. In countries where sanitary facilities are less comprehensive and undercooked or raw beef is eaten, *T. saginata* is highly prevalent. Examples include Kenya, Ethiopia, the Middle East, Yugoslavia, and parts of the former Soviet Union and South America.

Indigenously acquired disease rare in United States

## BEEF TAPEWORM DISEASE: CLINICAL ASPECTS

### MANIFESTATIONS

Most infected patients are asymptomatic and become aware of the infection only through the spontaneous passage of proglottids. The proglottids may be observed on the surface

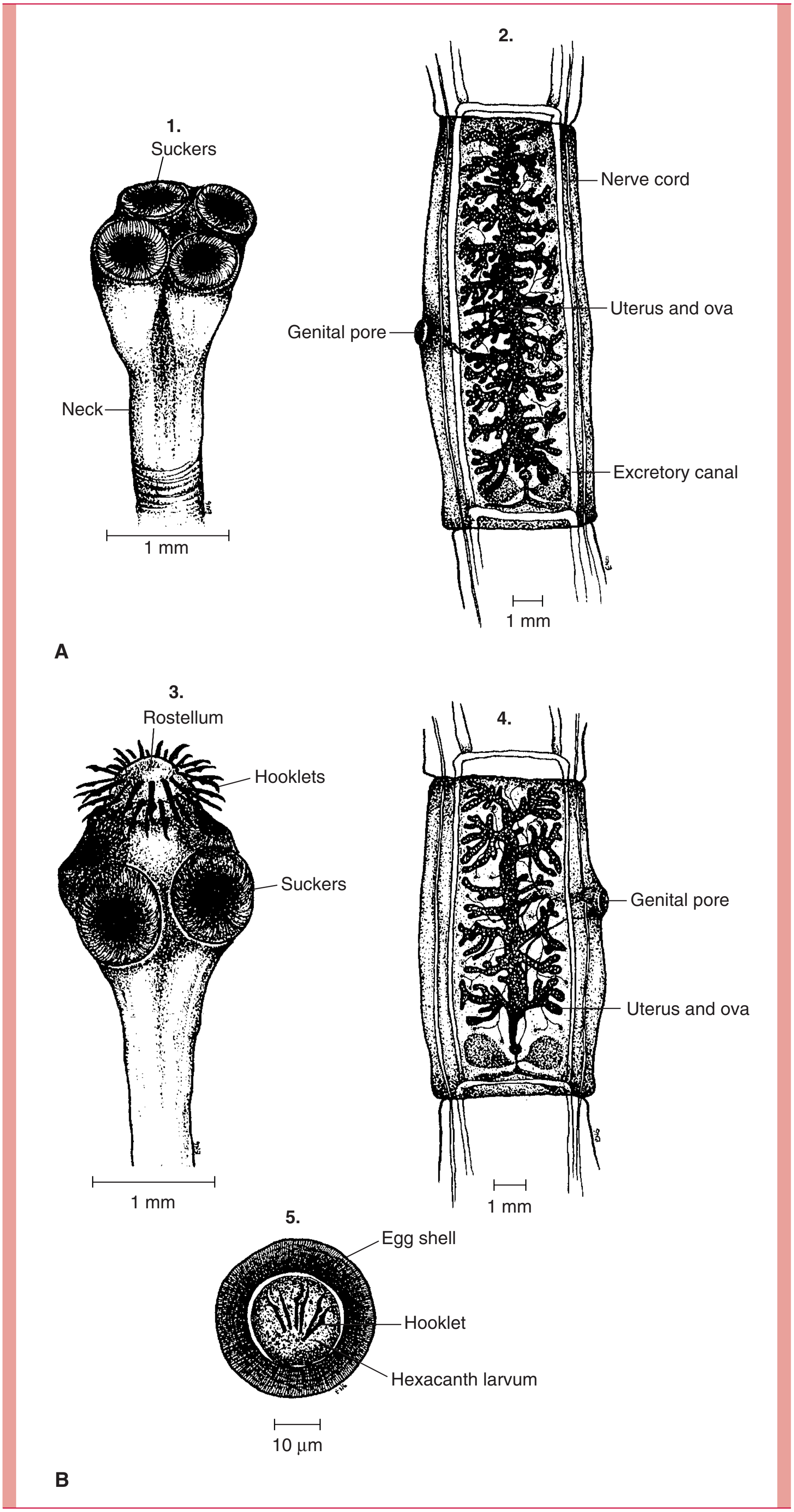


FIGURE 57–1

**A.** *Taenia saginata.* **B.** *Taenia solium.* (1, 3) Scolices; (2, 4) gravid proglottids; (5) ova (indistinguishable between species).

of the stool or appear in the underclothing or bed sheets of the alarmed host. Passage may occur very irregularly and can be precipitated by excessive alcohol consumption. Some patients report epigastric discomfort, nausea, irritability (particularly after passage of segments), diarrhea, and weight loss. Occasionally the proglottids may obstruct the appendix, biliary duct, or pancreatic duct.

Clinical symptoms usually mild

## DIAGNOSIS

The diagnosis is made by finding eggs or proglottids in the stool. Eggs may also be distributed on the perianal area secondary to rupture of proglottids during anal passage. The adhesive cellophane tape technique described for pinworm can be used to recover them from this area. With this procedure, 85 to 95% of infections are detected, in contrast to only 50 to 75% by stool examination. Because the eggs of *T. solium* and *T. saginata* are morphologically identical, it is necessary to examine a proglottid to identify the species correctly.

Adhesive cellophane tape technique and stool examination detect eggs and proglottids

## TREATMENT AND PREVENTION

The drugs of choice are praziquantel or niclosamide, which act directly on the worm. Both are highly effective in single-dose oral preparations. Ultimately, control is best effected through the sanitary disposal of human feces. Meat inspection is helpful; the cysticerci are readily visible. In areas where the infection is common, thorough cooking is the most practical method of control. Internal temperatures of 56°C or more for 5 minutes or longer destroy the cysticerci. Salting or freezing for 1 week at −15°C or less is also effective.

Sewage disposal, meat inspection, and adequate cooking

# PORK TAPEWORM

## *Taenia solium*: PARASITOLOGY

Like the beef tapeworm, which it closely resembles, *T. solium* inhabits the human jejunum, where it may survive for decades. It can be distinguished from its close relative only by careful scrutiny of the scolex and proglottids; *T. solium* possesses a rostellum armed with a double row of hooklets (Fig 57–1B3). The strobila is generally smaller than that of *T. saginata,* seldom exceeding 5 m in length or containing more than 1000 proglottids. Gravid segments measure 6 by 12 mm and thus appear less elongated than those of the bovine parasite (Fig 57–1B4). Typically, the uterus has only eight to twelve lateral branches. Although the eggs appear morphologically identical to those of *T. saginata,* they are infective only to swine and, perhaps reflecting a genetic proximity we would prefer to overlook, humans. Both pigs and people become intermediate hosts when they ingest food contaminated with viable eggs. Some authorities have suggested that humans may be autoinfected when gravid proglottids are carried backward into the stomach during the act of vomiting, initiating the release of the contained eggs. It seems more likely that autoinfection results from the transport of the eggs from the perianal area to the mouth on contaminated fingers.

*T. solium* strobila shorter than in *T. saginata*

Eggs infective to swine and to humans

Regardless of the route, an egg reaching the stomach of an appropriate intermediate host hatches, releasing the hexacanth embryo. The embryo penetrates the intestinal wall and may be carried by the lymphohematogenous system to any of the tissues of the body. Here it develops into a 1 cm, white, opalescent cysticercus over 3 to 4 months. The cysticercus may remain viable for up to 5 years, eventually infecting humans when they ingest undercooked and "measly" flesh. The scolex everts, attaches itself to the mucosa, and develops into a new adult worm, thereby completing the cycle.

Tissue cysticerci develop in humans and swine

# PORK TAPEWORM DISEASE

## EPIDEMIOLOGY

*T. solium* rarely found in United States

Although infected swine are still occasionally found in the United States, most human disease is found in immigrants from endemic areas. Although this infection is widely distributed throughout the world, it is particularly common in south and southeast Asia, Africa, Latin America, and Eastern Europe.

# PORK TAPEWORM DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

Major clinical manifestations caused by reaction to cysticerci

The signs and symptoms of infection with the adult worm are similar to those of taeniasis saginata. Clinical manifestations are totally different when humans serve as intermediate hosts. Cysticerci develop in the subcutaneous tissues, muscles, heart, lungs, liver, brain, and eye. As long as the number is small and the cysticerci remain viable, tissue reaction is moderate and the patient asymptomatic. The death of the larva, however, stimulates a marked inflammatory reaction, fever, muscle pains, and eosinophilia.

Meningoencephalitic syndrome with eosinophilia produced by CNS invasion

Multiple small cysts formed

Focal neurologic signs and epilepsy related to cysts

The most important and dramatic clinical presentation of cysticercosis results from lesions in the central nervous system (CNS). During the acute invasive stage, patients experience fever, headache, and eosinophilia. In heavy infections, a meningoencephalitic syndrome with cerebrospinal fluid (CSF) eosinophilic pleocytosis may be present. Established cysts can be found in the cerebrum, ventricles, subarachnoid space, spinal cord, or eye. Cerebral cysts are usually small, often measuring 2 cm or less in diameter; racemose lesions may be threefold larger. These parenchymal infections can induce focal neurologic abnormalities, personality changes, intellectual impairment and/or seizures; in many endemic areas, cysticercosis is the leading cause of epilepsy. Subarachnoid lesions and cysticerci located within the fourth ventricle may obstruct the flow of CSF, producing increased intracranial pressure with its associated headache, vomiting, visual disturbances, or psychiatric abnormalities. Multiple racemose lesions have a predilection for the basal cisterns, particularly in young women, from whence they rapidly spread around the base of the brain and cerebrum with catastrophic result. Spinal involvement produces cord compression or meningeal inflammation. Eye lesions incite pain and visual disturbances.

## DIAGNOSIS

Presence of adult worm diagnosed from proglottids

Biopsy required for cysticerci

Infection with the adult worm is diagnosed as described for *T. saginata.* Cysticercosis is suspected when an individual who has been in an endemic area presents with neurologic manifestations or subcutaneous nodules. Roentgenograms of the soft tissues often reveal dead, calcified cysticerci. Viable lesions may be detected as low-density masses by computed tomography (CT) or magnetic resonance imaging (MRI). Brain cysticerci typically are 5 to 10 mm in diameter. Subarachnoid lesions are often larger, may be lobulated, and are often "isodense," making them difficult to visualize radiographically. The diagnosis is confirmed by demonstrating the larva in a biopsy sample of a subcutaneous nodule or specific antibodies in the circulating blood. Serum and CSF enzyme immunoassays and Western blot testing for specific anticysticercal antibodies have a sensitivity of 80 to 95%. The presence of IgG antibodies alone may reflect the presence of past or inactive disease.

## TREATMENT AND PREVENTION

Infection with the adult worm is approached in the manner described for *T. saginata*. Because the mortality rate in patients with symptomatic neurocysticercosis approaches 50%, aggressive management is warranted. Patients with parenchymal lesions usually respond to prolonged treatment with praziquantel or albendazole. Concomitant corticosteroid administration helps minimize the inflammatory response to dying cysticerci. Intraventricular subarachnoid and eye lesions appear relatively refractory to chemotherapy; surgery, CSF shunts, and corticosteroids may help ameliorate symptoms.

Surgery occasionally needed for cysticercosis

# FISH TAPEWORM

## *Diphyllobothrium latum*: PARASITOLOGY

The adult *D. latum* attaches to the ileal mucosa with the aid of two sucking grooves (bothria) located in an elongated fusiform scolex (Fig 57–2). In lifespan and overall length, it resembles the *Taenia* species discussed previously. The 3000 to 4000 proglottids, however, are uniformly wider than they are long, accounting for this cestode's species designation as well as one of its common names, the broad tapeworm. The gravid segments contain a centrally positioned, rosette-shaped uterus unique among the tapeworms of humans. Unlike those of the *Taenia* species, ova are released through the uterine pore. Over 1 million oval (55 by 75 mm) operculate eggs are released daily into the stool (Fig 57–2B).

*D. latum* has broad proglottids

On reaching fresh water they hatch, releasing ciliated, free-swimming larvae or coracidia. If ingested within a few days by small freshwater crustaceans of the genera *Cyclops* or *Diaptomus,* they develop into procercoid larvae. When the crustacean is ingested by a freshwater or anadromous marine fish, the larvae migrate into the musculature of the fish and develop into infectious plerocercoid larvae. Humans are infected when they eat improperly prepared freshwater fish containing such forms.

Eggs release motile coracidia in water

Crustacean and fish intermediates; humans infected by ingesting inadequately cooked fish

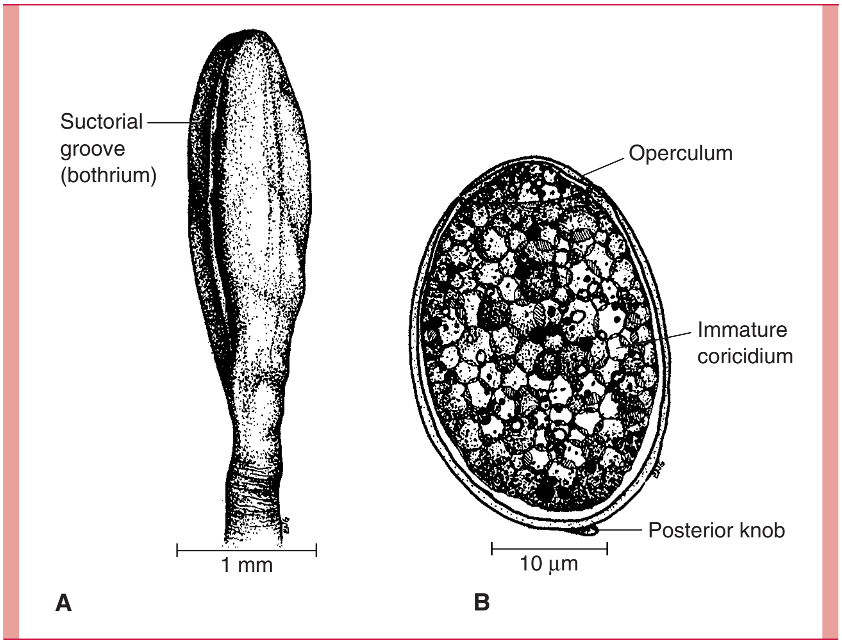


FIGURE 57–2
*Diphyllobothrium latum.*
**A.** Scolex. **B.** Ovum.

# FISH TAPEWORM DISEASE

## EPIDEMIOLOGY

Worldwide distribution

Worm found in Alaska, midwestern states, and Florida

Eating raw fish increases risk

Fish tapeworms are found wherever raw, pickled, or undercooked freshwater fish from fecally contaminated lakes and streams is eaten by humans. Other fish-eating mammals may also serve as reservoir hosts. Human infections have been described in the Baltic and Scandinavian countries, Russia, Switzerland, Italy, Japan, China, the South Pacific, Chile and Argentina. The worm, brought to North America by Scandinavian immigrants, is now found in Alaska, Canada, the midwestern states, California, and Florida. It was shown recently that infectious plerocercoid larvae may develop in anadromous salmon, and human cases have been traced to the ingestion of fish freshly taken from Alaskan waters. The increasing popularity of raw fish dishes such as Japanese sushi and sashimi may lead to increased prevalence of this disease in the United States. Among Ontario Indians, infection is acquired by eating fresh salted fish. Even when fish is appropriately cooked, individuals may become infected by sampling the flesh during the process of preparation.

# FISH TAPEWORM DISEASE: CLINICAL ASPECTS

## MANIFESTATIONS

Occasional intestinal obstruction

Vitamin $B_{12}$ deficiency related to consumption by worm

Most infected patients are asymptomatic. On occasion, however, they have complained of epigastric pain, abdominal cramping, vomiting, and weight loss. Moreover, the presence of several adult worms within the gut has been known to precipitate intestinal or biliary obstruction. Forty percent of fish tapeworm carriers demonstrate low serum levels of vitamin $B_{12}$, apparently as a result of the competition between the host and the worm for ingested vitamin. Studies have shown that a worm located high in the jejunum may take up 80 to 100% of vitamin $B_{12}$ given by mouth. Approximately 0.1 to 2% of patients develop macrocytic anemia. They tend to be elderly, to have impaired production of intrinsic factor, and to have worms located high in the jejunum. In many, folate absorption is also diminished. Lysolecithin, a tapeworm product, may also contribute to the anemia. Neurologic manifestations of vitamin $B_{12}$ deficiency occur, sometimes in the absence of anemia. They include numbness, paresthesia, loss of vibration sense, and, rarely, optic atrophy with central scotoma.

## DIAGNOSIS

Eggs demonstrated in stool

The diagnosis is established by finding the typical eggs in the stool. As *D. latum* produces large numbers of ova, identification is usually accomplished without the need for concentration techniques.

## TREATMENT AND PREVENTION

Fish rendered noninfectious at −10°C for 48 hours

Treatment is carried out as described for *T. saginata* infections. When anemia or neurologic manifestations are present, parenteral administration of vitamin $B_{12}$ is also indicated. Personal protection can be accomplished by thorough cooking of all salmon and freshwater fish. Devotees of raw fish may choose to freeze their favorite dish at −10°C for 48 hours before serving. Ultimately, control of diphyllobothriasis is accomplished only by prohibiting the discharge of untreated sewage into lakes and streams.

# ECHINOCOCCUS

Echinococcosis is a tissue infection of humans caused by larvae of *Echinococcus granulosus* and *E. multilocularis.* The former is a more common cause of human disease.

## *Echinococcus granulosus*

### PARASITOLOGY

The adult *E. granulosus* inhabits the small bowel of dogs, wolves, and other canines, where it survives for a scant 12 months. The scolex, like that of the genus *Taenia,* possesses four sucking discs and a double row of hooklets. The entire strobila, however, measures only 5 mm in length and contains but three proglottids; one immature, one mature, and one gravid. The latter segment splits either before or after passage in the stool, releasing eggs that appear identical to those of *T. saginata* and *T. solium.* A number of mammals may serve as intermediates, including sheep, goats, camels, deer, caribou, moose, and, most important, humans. When one of these hosts ingests eggs, they hatch. The embryos penetrate the intestinal mucosa and are carried by the portal blood to the liver. Here, many are filtered out in the hepatic sinusoids. The rest traverse the liver and are carried to the lung, where most lodge. A few pass through the pulmonary capillaries, enter the systemic circulation, and are carried to the brain, heart, bones, kidneys, and other tissues. Many of the larvae are phagocytosed and destroyed. The survivors form a cyst wall composed of an external laminated cuticle and an internal germinal membrane. The cyst fills with fluid and slowly expands, reaching a diameter of 1 cm over 5 to 6 months. Secondary or daughter cysts form within the original hydatid. Within each of these daughter cysts, new protoscolices are produced from the germinal lining. Some break free, dropping to the bottom of the cyst to form hydatid sand. When hydatid-containing tissues of the intermediate host are ingested by a canine, thousands of scolices are released in the intestine to develop into adult worms.

Adult in small intestine of canines

Herbivores and humans serve as intermediates

Larvae penetrate to portal or systemic circulation

Cysts and daughter cysts develop in tissues

Cycle completed with ingestion of cysts by canine

### ECHINOCOCCOSIS

#### EPIDEMIOLOGY

There are two major epidemiologic forms of *E. granulosus*–induced echinococcosis, pastoral and sylvatic. The more common pastoral form has its highest incidence in Australia, New Zealand, South and East Africa, the Middle East, Central Europe, and South America, where domestic herbivores such as sheep, cattle, and camels are raised in close contact with dogs. Although approximately 200 human cases are reported each year in the United States, most were acquired elsewhere. Indigenous cases do occur, however, particularly among Basque sheep farmers in California, southwestern Native Americans, and some Utah shepherds. Animal husbandry practices that permit dogs to feed on the raw viscera of slaughtered sheep allow the cycle of transmission to continue. Shepherds become infected while handling or fondling their dogs. Eggs retained in the fur of these animals are picked up on the hands and later ingested. Sylvatic echinococcosis is found principally in Alaska and western Canada, where wolves act as the definitive host and

Pastoral infections maintained by allowing dogs to feed on sheep viscera

Hand-to-mouth infection of humans by dog contact

Sylvatic cycle in Alaska and western Canada moose or caribou as the intermediate. In two counties in California, a second cycle involving deer and coyotes has been described. When hunters kill these wild deer and feed their offal to accompanying dogs, a pastoral cycle may be established.

# ECHINOCOCCOSIS: CLINICAL ASPECTS

## MANIFESTATIONS

Disease caused by mechanical effects of cysts after many years

The enlarging *E. granulosis* cysts produce tissue damage by mechanical means. The clinical presentation depends on their number, site, and rate of growth. Typically, there is a latent period of 5 to 20 years between acquisition of infection and subsequent diagnosis. Intervals as long as 75 years have been reported occasionally.

Pulmonary cysts predominate in sylvatic disease, hepatic in pastoral

Cysts may attain large size

Rupture leads to hypersensitivity manifestations and dissemination

In sylvatic infections, two thirds of the cysts are found in the lung, the remainder in the liver. Most patients are asymptomatic when the lesion is discovered on routine chest x-ray or physical examination. Occasionally, the patient may present with hemoptysis, pain in the right upper quadrant of the abdomen, or a tender hepatic mass. Significant morbidity is uncommon, and death extremely rare. In the pastoral form of disease, 60% of the cysts are found in the liver, 25% in the lung. One fifth of all patients show involvement of multiple sites. The hydatid cysts, which grow more rapidly (0.25 to 1 cm/year) than the sylvatic lesions, may reach enormous size. Twenty percent eventually rupture, inducing fever, pruritus, urticaria, and, at times, anaphylactic shock and death. Release of thousands of scolices may lead to dissemination of the infection. Rupture of pulmonary lesions also induces cough, chest pain, and hemoptysis. Liver cysts may break through the diaphragm or rupture into the bile duct or peritoneal cavity. The majority, however, present as a tender, palpable hepatic mass. Intrabiliary extrusion of calcified cysts may mimic the signs of acute cholecystitis; complete obstruction results in jaundice. Bone cysts produce pathologic fractures, whereas lesions in the CNS are often manifest as blindness or epilepsy. Cardiac lesions have been associated with conduction disturbances, ventricular rupture, and embolic metastases. It has been suggested that circulating antigen–antibody complexes may be deposited in the kidney, initiating membranous glomerulonephritis.

## DIAGNOSIS

Radiologic and scanning appearance characteristic

In *E. granulosis*–infected patients, chest x-rays reveal pulmonary lesions as slightly irregular, round masses of uniform density devoid of calcification. In contrast, more than one half of hepatic lesions display a smooth, calcific rim. CT, ultrasonography, and MRI may reveal either a simple fluid-filled cyst or daughter cysts with hydatid sand. Endoscopic retrograde cholangiography has been valuable for determining cyst location and possible communication with the biliary tree. Because of the potential for an anaphylactoid reaction and dissemination of infection, diagnostic aspiration has been considered contraindicated. Nevertheless, in the hands of some investigators, ultrasonically guided percutaneous drainage, followed by the introduction of ethanol to kill protoscoleces and germinal layer, has proven to be safe and useful, both diagnostically and therapeutically (see below). In patients with ruptured pulmonary cysts, scolices may be demonstrated in the sputum.

Serologic diagnosis important but needs improved sensitivity

In most cases, confirmation of the diagnosis requires serologic testing. Unfortunately, current procedures are not totally satisfactory. Indirect hemagglutination and latex agglutination tests are positive in 90% of patients with hepatic lesions and 60% of those with pulmonary hydatid cysts. When using hydatid cyst fluid or soluble scolex antigen, the presence of a precipitin line in the immunoelectrophoresis test appears to be more specific. An adaption of this test to an enzyme-linked immunoelectrodiffusion technique appears to provide a rapid, sensitive diagnostic test. Other serologic tests are in the process of evaluation. Polymerase chain reaction assay has been shown capable of detecting picogram quantities of *Echinococcus* genomic DNA in fine-needle biopsy material from patients with suspected echinococcosis.

## TREATMENT AND PREVENTION

For years, the only definitive therapy available was surgical extirpation. Patients with pulmonary hydatid cysts of the sylvatic type and small calcified hepatic lesions underwent surgery only when they became symptomatic or the cysts increased dramatically in size over time. For other lesions, **P**ercutaneous **A**spiration, **I**nfusion of scolicidal and **R**easpiration (PAIR) can be utilized in lieu of surgery. Presently, it is recommended that high-dose albendazole be administered prior to, and for several weeks (or years in the case of *E. multilocularis* infection) after surgery and/or aspiration. Infected dogs should be wormed, and infected carcasses and offal burned or buried. Hands should be carefully washed after contact with potentially infected dogs.

Treatment may include careful aspiration with concomitant albendazole

# *Echinococcus multilocularis*

*E. multilocularis* is found primarily in subarctic and arctic regions in North America, Europe, and Asia. The adult worms are found in the gut of foxes and, to a lesser extent, coyotes. Their larval forms find harborage in the tissues of mice and voles, the canines' rodent prey. Domestic dogs may acquire adult tapeworms by killing and ingesting these larval-infected sylvatic rodents. Humans are infected with larval forms through the ingestion of eggs passed in the feces of their domestic dogs or ingestion of egg-contaminated vegetation. Unlike the larval forms of *E. granulosis,* those of *E. multilocularis* bud externally, producing proliferative, multilocular cysts that slowly but progressively invade and destroy the affected organs and adjacent tissues.

Larvae bud externally; produce multilocular cysts

The clinical course in humans is characterized by epigastric pain; obstructive jaundice; and, less frequently, metastasis to the lung and brain, thus closely mimicking a hepatoma. Serologic tests are usually positive. Combined drug and surgical treatment often slows the progress of the disease and relieves symptoms. It is seldom curative.

## ADDITIONAL READING

Bandres JC, White AC Jr, Samo T, et al. Extraparenchymal neurocysticercosis: Report of five cases and review of management. *Clin Infect Dis* 1992;15:799–811. A very good review of treatment of these refractory forms of neurocysticercosis.

Filice C, Di Perri G, Strosselli M, et al. Parasitologic findings in percutaneous drainage of human hydatid liver cysts. *J Infect Dis* 1990;161:1290–1295. Authors demonstrate that, when appropriately performed, diagnostic and therapeutic aspiration of echinococcal cysts is safe and effective.

Franchi C, et al. Long-term evaluation of patients with hydatidosis treated with benzimidazole carbamates. *Clin Infect Dis* 1999;29:304.

Khuroo MS, et al. Percutaneous drainage compared with surgery for hepatic hydatid cysts. *N Engl J Med* 1997;337:881.

Ruttenber AJ, Weniger BG, Sorvillo F, et al. Diphyllobothriasis associated with salmon consumption in Pacific Coast states. *Am J Trop Med Hyg* 1984;33:455–459.

Shantz PM, Kramer HJ. Larval cestode infections: cysticercosis and echinococcosis. *Curr Opin Infect Dis* 1995;8:32.

Schantz PM, Moore AC, Munoz JL, et al. Neurocysticercosis in an orthodox Jewish community in New York City. *N Engl J Med* 1992;327:692–701. An important paper that demonstrates that cysticercosis can be readily acquired from food-handlers infected with the adult tapeworm. Emigrants from countries endemic for *T. solium* infection should be screened for tapeworm infection before they are employed as housekeepers or food handlers.

White AC Jr. Neurocysticercosis: a major cause of neurologic disease worldwide. *Clin Infect Dis* 1997;24:101.

CHAPTER 58

# Trematodes

JAMES J. PLORDE

Of the myriad relationships that have developed between helminths and humans over the millennia of our mutual existence, none has proved more destructive to our health and productivity than that forged with the indomitable flukes. Typically, the adults live for decades within human tissues and vascular systems, where they resist immunologic attack and produce progressive damage to vital organs. Morphologically, trematodes are bilaterally symmetric, vary in length from a few millimeters to several centimeters, and possess two deep suckers from which they derive their name ("body with holes"). One surrounds the oral cavity, and the other is located on the ventral surface of the worm. These organs are used for both attachment and locomotion; movement is effected in a characteristic inchworm fashion. The digestive tract begins at the oral sucker and continues as a muscular pharynx and esophagus before bifurcating to form bilateral ceca that end blindly near the posterior extremity of the worm. Undigested food is vomited out through the oral cavity. The excretory system consists of a number of hollow, ciliated flame cells that excrete waste products into interconnecting ducts terminating in a posterior excretory pore.

Persistent flukes move through tissue and vasculature with inchworm locomotion

The reproductive systems vary and serve as a means for dividing the trematodes into two major categories: the hermaphrodites and the schistosomes. The adult hermaphrodite contains both male and female gonads and produces operculate eggs. The schistosomes have separate sexes, and the fertilized female deposits only nonoperculated offspring. The two groups have similar life cycles. The major differential features are summarized in Table 58–1. Eggs are excreted from the human host and, if they reach fresh water, hatch to release ciliated larvae called miracidia. These larvae find and penetrate a snail host specific for the trematode species. In this intermediate host, they are transformed by a process of asexual reproduction into thousands of tail-bearing larvae or cercariae, which are released from the snail over a period of weeks and swim about vigorously in search of their next host. In the case of schistosomal cercariae, this host is the human. When they come in contact with the skin surface, they attach, discard their tails, and invade, thereby completing their life cycle. The cercariae of the hermaphroditic flukes encyst in or on an aquatic plant or animal, where they undergo a second transformation to become infective metacercariae. Their cycle is completed when the second intermediate host is ingested by a human.

Two types of reproductive systems

Snails release motile cercariae in water

Of the many trematodes that infect humans, only five are discussed: the blood flukes, all of which are members of the genus *Schistosoma* (*S. mansoni, S. haematobium,* and *S. japonicum*); and the lung (*Paragonimus* spp.) and liver (*Clonorchis sinensis*) flukes, which are hermaphroditic (Fig 58–1). Basic details of other hermaphroditic tissue and intestinal flukes are listed in Table 58–2.

*Schistosoma* cercariae infect humans through skin

*Paragonimus* and *Clonorchis* have second intermediate host

TABLE 58–1

## General Characteristics of Trematodes

| | Trematode Type | |
|---|---|---|
| Characteristic | Blood | Tissue/Intestinal |
| Genus | *Schistosoma* | *Paragonimus, Clonorchis, Opisthorchis, Fasciola* |
| Morphology | | |
| Adult | Oral and ventral suckers<br>Blind gastrointestinal tract<br>Slender, worm-like | Oral and ventral suckers<br>Blind gastrointestinal tract<br>Flat, leaf-like |
| Egg | Nonoperculate | Operculate |
| Biology | | |
| Sexes | Separate | Hermaphroditic |
| Intermediates | One | Two |
| Life span | Long | Long |

FIGURE 58–1
Adult flukes and eggs.

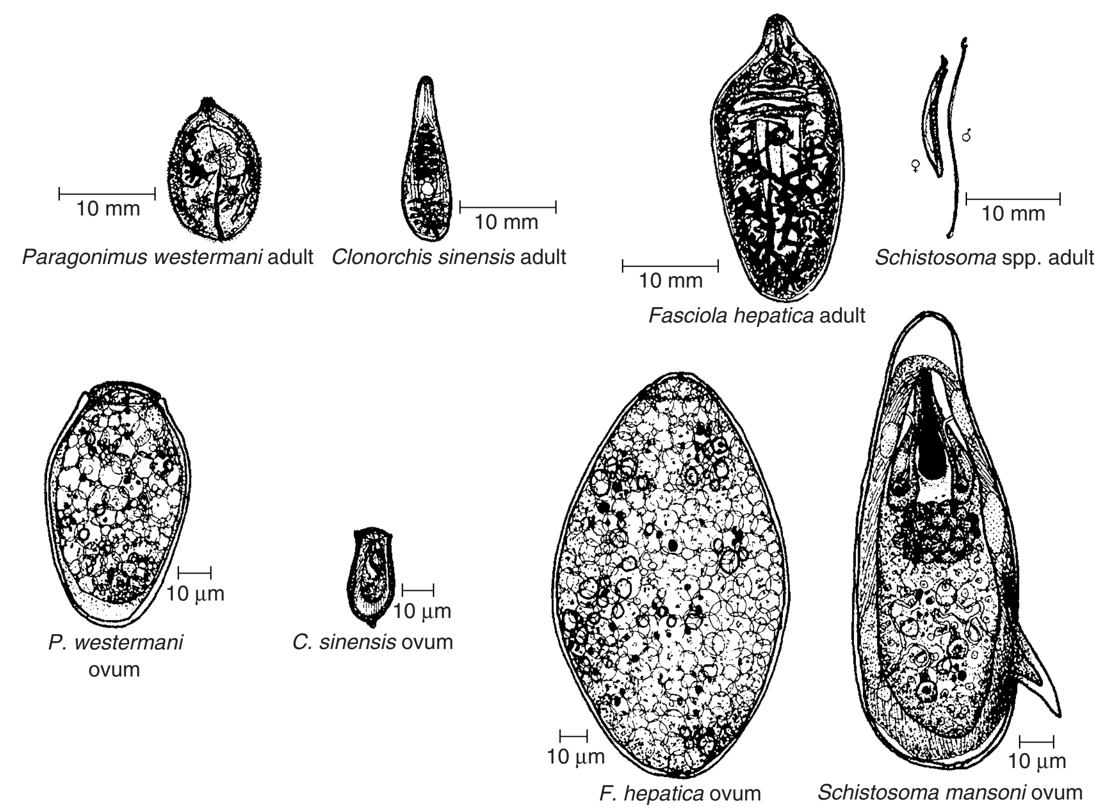

TABLE 58–2

**Intestinal and Tissue Trematodes**

| | *Paragonimus* | *Clonorchis* | *Opisthorchis* | *Fasciola* | *Fasciolopsis* | *Heterophyes/ Metagonimus* |
|---|---|---|---|---|---|---|
| **Distribution** | | | | | | |
| Geographic | Asia, Africa, Central America | Japan, China, Taiwan, Vietnam | Asia, Eastern Europe | Worldwide | East and Southeast Asia | Asia, former USSR, Mediterranean |
| Infected population (in millions) | 3 | 20 | 4 | – | 10 | – |
| **Adult Worms** | | | | | | |
| Reservoir hosts | Domestic and wild animals | Cats, dogs | Domestic and wild animals | Sheep and other herbivores | Pigs | Fish-eating mammals |
| Location in body | Lungs, CNS | Biliary tract | Biliary tract | Biliary tract | Small intestine | Small intestine |
| Length (mm) | 7–12 | 10–25 | 10 | 20–30 | 20–75 | 1–2 |
| Life span (years) | 4–6 | 20–30 | 20–30 | 10–15 | 0.5 | 1 |
| **Eggs** | | | | | | |
| Characteristics | Operculated | Operculated | Operculated | Operculated | Operculated | Operculated |
| Size ($\mu$m) | 80–100 | 26–30 | 26–30 | 130–150 | 130–150 | 26–30 |
| Location[a] | Sputum, stool | Bile, stool | Bile, stool | Bile, stool | Stool | Stool |
| **Larvae** | | | | | | |
| First intermediate | Snail | Snail | Snail | Snail | Snail | Snail |
| Second intermediate | Freshwater crab and crayfish | Freshwater fish | Freshwater fish | Watercress and other aquatic plants | Water chestnut and other aquatic plants | Freshwater fish |

*Abbreviation:* CNS = central nervous system.

[a] Diagnostic specimens.

# Paragonimus

## PARAGONIMUS SPECIES: PARASITOLOGY

Several *Paragonimus* species may infect humans. *P. westermani,* which is widely distributed in East Asia, is the species most frequently involved. The short, plump (10 by 5 mm), reddish-brown adults are characteristically found encapsulated in the pulmonary parenchyma of their definitive host. Here they deposit operculate, golden-brown eggs, which are distinguished from similar structures by their size (50 by 90 $\mu$m) and prominent periopercular shoulder. When the capsule erodes into a bronchiole, the eggs are coughed up and spat out or swallowed and passed in the stool. If they reach fresh water, they embryonate several weeks before the ciliated miracidia emerge through the open opercula. After invasion of an appropriate snail host, 3 to 5 months pass before cercariae are released. These larval forms invade the gills, musculature, and viscera of certain crayfish or freshwater crabs; over 6 to 8 weeks, the larval forms transform into metacercariae. When the raw or undercooked flesh of the second intermediate host is ingested by humans, the metacercariae encyst in the duodenum and burrow through the gut wall into the peritoneal cavity. The majority continue their migration through the diaphragm and reach maturity in the lungs 5 to 6 weeks later. Some organisms, however, are retained in the

Adults encapsulate in lung

Capsule erodes into bronchiole and eggs are coughed up; cycle continues if eggs reach water with susceptible snail

Crayfish and freshwater crabs are second intermediate hosts

Other carnivores are also definitive hosts intestinal wall and mesentery or wander to other foci such as the liver, pancreas, kidney, skeletal muscle, or subcutaneous tissue. Young worms migrating through the neck and jugular foramen may encyst in the brain, the most common ectopic site. In addition to humans, other carnivores, including the rat, cat, dog, and pig, may serve as definitive hosts. Immature ectopic adults in the striated muscles of the pig may infect humans after ingestion of undercooked pork.

## PARAGONIMIASIS (LUNG FLUKE INFECTION)

### EPIDEMIOLOGY

Infected snails often found in mountain streams

Humans infected by ingesting infected crustaceans

Although most of the 5 million human infections are concentrated in the Far East (eg, Korea, Japan, China, Taiwan, the Philippines, and Indonesia), paragonimiasis has recently been described in India, Africa (*P. africanus*), and Latin America (*P. mexicanus*). *P. kellicotti,* a parasite of mink, is widely distributed in eastern Canada and the United States but rarely produces human infection. Approximately 1% of recent Indochinese immigrants to the United States are found to be infected with *P. westermani.* Infection of the snail host, which is typically found in small mountain streams located away from human habitation, is probably maintained by animal hosts other than humans. Human disease occurs when food shortages or local customs expose individuals to infected crabs. When these crustaceans are prepared for cooking, juice containing metacercariae may be left behind on the working surface and contaminate other foods subsequently prepared in the same area. Fresh crab juice, which is used for the treatment of infertility in Cameroon and of measles in Korea, may also transmit the disease. In the Far East, crabs are frequently eaten after they have been lightly salted, pickled, or immersed briefly in wine (drunken crab), practices that are seldom lethal to the metacercariae. Children living in endemic areas may be infected while handling or ingesting crabs during the course of play.

## PARAGONIMIASIS (LUNG FLUKE INFECTION): CLINICAL ASPECTS

### MANIFESTATIONS

Multiple lung cysts are formed

Secondary infection of ruptured cysts produces bronchitis

Chronic pulmonary abscess may resemble tuberculosis

The presence of the adult worms in the lung elicits an eosinophilic inflammatory reaction and, eventually, the formation of a 1- to 2-cm fibrous capsule that surrounds and encloses one or more parasites. The infected patient may harbor as many as 25 such lesions. With the onset of oviposition, the capsule swells and erodes into a bronchiole, resulting in expectoration of the brownish eggs, blood, and an inflammatory exudate. Secondary bacterial infection of the evacuated cysts is common, producing a clinical picture of chronic bronchitis or bronchiectasis. When cysts rupture into the pleural cavity, chest pain and effusion can result. Early in infection, chest x-rays demonstrate small segmental infiltrates; these are gradually replaced by round nodules that may cavitate. Eventually, cystic rings, fibrosis, and calcification occur, producing a picture closely resembling that of pulmonary tuberculosis. The confusion is compounded by the frequent coexistence of the two diseases.

Adult flukes in the intestine and mesentery produce pain, bloody diarrhea, and on occasion, palpable abdominal or cutaneous masses; the latter is characteristic of a second Chinese fluke, *P. skrjiabini.* In approximately 1% of cases in the Far East, more commonly in children, parasites lodge in the brain and produce a variety of neurologic manifestations, including epilepsy, paralysis, homonymous hemianopsia, optic atrophy, and papilledema.

## DIAGNOSIS

Eggs are usually absent from the sputum during the first 3 months of overt infection; however, repeated examinations eventually demonstrate them in more than 75% of infected patients. When a pleural effusion is present, it should be checked for eggs. Stool examination is frequently helpful, particularly in children who swallow their expectorated sputum. Approximately 50% of patients with brain lesions demonstrate calcification on x-ray films of the skull. The cerebrospinal fluid in such cases shows elevated protein levels and eosinophilic leukocytosis. A diagnosis in these cases, however, often depends on the detection of circulating antibodies. Their presence usually correlates well with acute disease and disappears with successful therapy. Recently developed antigen detection techniques have been proven to be both highly sensitive and specific and may soon displace antibody detection procedures.

Eggs difficult to find in sputum, pleural fluid, and feces

Serodiagnosis, antigen detection procedures available

## TREATMENT AND PREVENTION

The disease responds well to praziquantel or bithionol therapy. Control requires adequate cooking of shellfish before ingestion.

# CLONORCHIS

## *Clonorchis sinensis:* PARASITOLOGY

Flukes of the genera *Fasciola, Opisthorchis,* and *Clonorchis* may all infect the human biliary tract and at times produce manifestations of ductal obstruction. *C. sinensis,* the Chinese liver fluke, is the most important and is discussed here (see Table 58–2). The small, slender (5 by 15 mm) adult survives up to 50 years in the biliary tract of its host by feasting on the rich mucosal secretions. A cone-shaped anterior pole, a large oral sucker, and a pair of deeply lobular testes arranged one behind the other in the posterior third of the worm distinguish it from other hepatic parasites. Approximately 2000 tiny (15 by 30 $\mu$m) ovoid eggs are discharged daily and find their way down the bile duct and into the fecal stream. The exquisite urn-shaped shells have a discernible shoulder at their opercular rim and a tiny knob on the broader posterior pole. On reaching fresh water, they are ingested by their intermediate snail host, transformed into cercariae, and released to penetrate the tissues of freshwater fish, in which they encyst to form metacercariae. If the latter host is ingested by a fish-eating mammal, the larvae are released in the duodenum, ascend the common bile duct, migrate to the second-order bile ducts, and mature to adulthood over 30 days.

In addition to humans, rats, cats, dogs, and pigs may serve as definitive hosts.

Adults survive decades in biliary tract

Eggs discharged in bile ducts appear in feces

Snails are first intermediate host and fish second

Metacercariae from ingested fish migrate to biliary system

## CLONORCHIASIS (LIVER FLUKE INFECTION)

## EPIDEMIOLOGY

Clonorchiasis is endemic in the Far East, particularly in Korea, Japan, Taiwan, the Red River Valley of Vietnam, the Southern Chinese province of Kwantung, and Hong Kong. In previous years, parasite transmission was perpetuated by the practice of fertilizing commercial fish ponds with human feces. Recent improvements in the disposal of human waste have diminished acquisition of the disease in most countries. However, the

Endemic in Far East

Transmission to humans related to waste disposal

Ingestion of uncooked fish infects humans

extremely long lifespan of these worms is reflected in a much slower decrease in the overall infection rate. In some villages in southern China, the entire adult population is infected. A recent survey of stool specimens from immigrants from Hong Kong to Canada showed an infection rate of more than 15% overall and 23% in adults between 30 and 50 years of age. The disease is acquired by eating raw, frozen, dried, salted, smoked, or pickled fish. Commercial shipment of such products outside of the endemic area may result in the acquisition of worms far from their original source.

## CLONORCHIASIS (LIVER FLUKE INFECTION)

### MANIFESTATIONS

Light infection usually asymptomatic

Severe hepatic and biliary manifestations from heavy worm loads

Migration of the larvae from the duodenum to the bile duct may produce fever, chills, mild jaundice, eosinophilia, and liver enlargement. The adult worm induces epithelial hyperplasia, adenoma formation, and inflammation and fibrosis around the smaller bile ducts. In light infection, clinical disease seldom results. However, numerous reinfections may produce worm loads of 500 to 1000, resulting in the formation of bile stones and sometimes bile duct carcinoma in patients with severe, long-standing infections. Calculus formation is often accompanied by asymptomatic biliary carriage of *Salmonella typhi.* Dead worms may obstruct the common bile duct and induce secondary bacterial cholangitis, which may be accompanied by bacteremia, endotoxin shock, and hypoglycemia. Occasionally, adult worms are found in the pancreatic ducts, where they can produce ductal obstruction and acute pancreatitis.

### DIAGNOSIS

Distinctive eggs present in feces and duodenal aspirates

Eosinophilia common in acute disease

Definitive diagnosis requires the recovery and identification of the distinctive egg from the stool or duodenal aspirates. In mild infections, repeated examinations may be required. Because most patients are asymptomatic, any individual with clinical manifestations of disease in whom *Clonorchis* eggs are found must be evaluated for the presence of other causes of illness. In acute symptomatic clonorchiasis, there is usually leukocytosis, eosinophilia, elevation of alkaline phosphatase levels, and abnormal computed tomography and ultrasonographic liver scans. Cholangiograms may reveal dilatation of the intrahepatic ducts, small filling defects compatible with the presence of adult worms, and occasionally cholangiocarcinoma.

### TREATMENT AND PREVENTION

Praziquantel and albendazole have proven to be effective therapeutic agents. Prevention requires thorough cooking of freshwater fish and sanitary disposal of human feces.

# *SCHISTOSOMA*

## *SCHISTOSOMA* SPECIES: PARASITOLOGY

The schistosomes are a group of closely related flukes that inhabit the portal vascular system of a number of animals. Of the five species known to infect humans, three, *S. mansoni, S. haematobium,* and *S. japonicum,* are of primary importance. They infect 200 to

300 million individuals in Africa, the Middle East, Southeast Asia, the Caribbean and South America, and kill 1 million annually. The remaining two species are found in limited areas of West Africa (*S. intercalatum*) and Southeast Asia (*S. mekongi*), and will not be discussed in detail.

Inhabit portal vascular system

The adult worms can be distinguished from the hermaphroditic trematodes by the anterior location of their ventral sucker, by their cylindric bodies, and by their reproductive systems (ie, separate sexes). They are differentiated from one another only with difficulty. The 1- to 2-cm male possesses a deep ventral groove, or gynecophoral canal, in which it carries the longer, more slender female in lifelong copulatory embrace. After mating in the portal vein, the conjoined couple use their suckers to ascend the mesenteric vessels against the flow of blood. Guided by unknown stimuli, *S. japonicum* enters the superior mesenteric vein, eventually reaching the venous radicals of the small intestine and ascending colon; *S. mansoni* and *S. haematobium* are directed to the inferior mesenteric system. The destination of the former is the descending colon and rectum; the latter, however, passes through the hemorrhoidal plexus to the systemic venous system, ultimately coming to rest in the venous plexus of the bladder and other pelvic organs.

Different morphology and separate sexes

*S. mansoni* reaches colon and rectum and *S. haematobium* reaches veins of bladder and pelvic organs

On reaching the submucosal venules, the worms initiate oviposition. Each pair deposits 300 (*S. mansoni, S. haematobium*) to 3000 (*S. japonicum*) eggs daily for the remainder of its 4- to 35-year life span. Enzymes secreted by the enclosed miracidium diffuse through the shell and digest the surrounding tissue. Ova lying immediately adjacent to the mucosal surface rupture into the lumen of the bowel (*S. mansoni, S. japonicum*) or bladder (*S. haematobium*) and are passed to the outside in the excreta. Here, with appropriate techniques, they may be readily observed and differentiated. The eggs of *S. mansoni* are oval, possess a sharp lateral spine, and measure 60 by 140 $\mu$m. Those of *S. haematobium* differ primarily in the terminal location of their spine. The eggs of *S. japonicum,* in contrast, are more nearly circular, measuring 70 by 90 $\mu$m. A minute lateral spine can be visualized only with care.

Eggs deposited submucosally, rupture to lumina, and pass outside

When the eggs are deposited in fresh water, the miracidia hatch quickly. On finding a snail host appropriate for their species, they invade and are transformed over 1 to 2 months into thousands of forked-tailed cercariae. When released from the snail, these infectious larvae swim about vigorously for a few days. Cercariae coming in contact with human skin during this time attach, discard their tails, and penetrate. During a 1- to 3-day sojourn in the skin, the outer cercarial membrane is transformed from a trilaminar to a heptalaminar structure, an adaption that is thought to be critical to the survival of the parasite within the human body. The resulting schistosomula enter small venules and find their way through the right side of the heart to the lung. After a delay of several days, the parasites enter the systemic circulation and are distributed to the gut. Those surviving passage through the pulmonary and intestinal capillary beds return to the portal vein, where they mature to sexually active adults over 1 to 3 months.

In water eggs hatch to form miracidia, which invade snail

Cercariae from snail traverse human skin and vascular system

## SCHISTOSOMIASIS (BLOOD FLUKE INFECTION)

### EPIDEMIOLOGY

The widespread distribution and extensive morbidity of schistosomiasis makes it the single most important helminthic infection in the world today. Currently, more than 200 million individuals in 74 countries are infected. The continued presence of the parasite depends on the disposal of infected human excrement into fresh water, the availability of appropriate snail hosts, and the exposure of humans to water infected with cercariae. The construction of modern sanitation and water purification facilities would break this cycle of transmission but exceeds the economic resources of most endemic nations. Paradoxically, several massive land irrigation projects launched over the past two decades for the express purpose of speeding economic development have resulted in the dispersion of infected humans and snails to previously uninvolved areas. *S. mansoni,* the most

Most important of helminthic infections would be stopped by modern waste disposal

Spread to areas caused by new irrigation projects widespread of the blood flukes, is the only one present in the Western Hemisphere. Originally thought to have been introduced by African slaves, it is now found in Venezuela, Brazil, Surinam, Puerto Rico, the Dominican Republic, St. Lucia, and several other Caribbean islands.

Geographic distribution varies with species and depends on presence of snail host

Because a suitable snail host is lacking, transmission does not occur within the continental United States; however, nearly half a million individuals residing there have acquired schistosomiasis elsewhere. Puerto Rican, Yemenite, and Southeast Asian populations are those predominantly involved. In the Eastern Hemisphere, the prevalence of *S. mansoni* infection is highest in the Nile Delta and the tropical section of Africa. Isolated foci are also found in East and South Africa, Yemen, Saudi Arabia, and Israel.

*S. haematobium* is largely confined to Africa and the Middle East, where its distribution overlaps that of *S. mansoni. Schistosoma japonicum* affects the agricultural populations of several Far Eastern countries, including Japan, China, the Philippines, and the Celebes. The closely related *S. mekongi* is found in the Mekong and Mun River valleys of Vietnam, Thailand, Cambodia, and Laos.

Age-related susceptibility with peak in second decade

Within endemic areas, there are wide variations in both infection rates and worm loads. In general, both peak in the second decade of life and then decrease with advancing age. This finding has been explained in part by changes in the intensity of water exposure and in part by the slow development of IgE-mediated immunity. Most infected patients carry fewer than 10 pairs of worms in the vascular system and, accordingly, lack clinical manifestations of disease. Individuals who develop much heavier loads as a result of repeated infections may experience serious morbidity or mortality. Patients with concomitant *S. mansoni* and human immunodeficiency virus infections excrete substantially fewer eggs in their stool.

## PATHOGENESIS

There are three major clinicopathologic stages in schistosomiasis. The first stage is initiated by the penetration and migration of the schistosomula. The second or intermediate stage begins with oviposition and is associated with a complex of clinical manifestations. The third or chronic stage is characterized by granuloma formation and scarring around retained eggs.

## IMMUNITY

Major manifestations from cell-mediated immune response to eggs

The major clinicopathologic manifestations of schistosomiasis result from the host's cell-mediated immune response to the presence of retained eggs. With time, the intensity of this reaction is muted; granulomas formed in the later stages of infection are smaller and less damaging than those formed early. The mechanisms responsible for this modulation are not fully understood. Present evidence suggests that both suppressor T lymphocyte activity and antibody blockade are involved. The correlation in humans between HLA types A1 and B5 and the development of hepatosplenomegaly suggests that the extent of the immunoregulation is influenced, at least in part, by the genetic background of the host.

Blocking antibodies and adsorption of host molecules provide antigenic disguise

Concomitant immunity prevents new infections

As evidenced by their prolonged survival, the adult worms are remarkably well tolerated by their hosts. In part, this tolerance may be attributable to the formation of IgG4 blocking antibodies early in the course of infection. Tolerance may also reflect the ability of the developing parasites to disguise themselves by adsorbing host molecules, including immunoglobulins, blood group glycolipids, and histocompatibility complex antigens. Nevertheless, as mentioned earlier, the prevalence and intensity of human infection begins to abate during adolescence, despite continuing exposure to infective cercariae. It has been suggested that schistosomula penetrating the skin after the primary infection are coated with specific antibody, bound to eosinophils, and destroyed before they can reach the portal system. Although protection is not complete, the 60 to 80% kill rate is highly effective in controlling the intensity of parasitism. This condition, in which adult worms from a primary infection can survive in a host resistant to reinfection, has been termed concomitant immunity. Eventually, production of blocking antibodies wanes and that of protective IgE antibodies active against adult worms increases, leading to a decrease in the host's total worm population.

# SCHISTOSOMIASIS (BLOOD FLUKE INFECTION): CLINICAL ASPECTS

## Early Stage

Within 24 hours of penetrating the skin, a large proportion of the schistosomula die. In *S. mansoni* and *S. haematobium* infections, immediate and delayed hypersensitivity to parasitic antigens results in an intensely pruritic papular skin rash that increases in severity with repeated exposures to cercariae. As the viable schistosomula begin their migration to the liver, the rash disappears and the patient experiences fever, headache, and abdominal pain for 1 to 2 weeks.

Local and systemic hypersensitivity reactions produce rash

**Note:** In the United States, cercariae of avian schistosomes can penetrate human skin and die, producing an intensely pruritic, transient rash known as "swimmers' itch." No further disease occurs.

## Intermediate Stage

One to two months after primary exposure, patients with severe *S. mansoni* or *S. japonicum* infections may experience the onset of an acute febrile illness that bears a striking resemblance to serum sickness. The onset of oviposition leads to a state of relative antigen excess, the formation of soluble immune complexes, and the deposition of these in the tissues of the host. Indeed, high levels of such complexes have been demonstrated in the peripheral blood and correlate well with the severity of illness. In addition to the fever and chills, patients experience cough, urticaria, arthralgia, lymphadenopathy, splenomegaly, abdominal pain, and diarrhea. Sigmoidoscopic examination reveals an inflamed colonic mucosa and petechial hemorrhages; occasionally, patients with *S. japonicum* infection develop clinical manifestations of encephalitis. Typically, leukocytosis; marked peripheral eosinophilia; and elevated levels of IgM, IgG, and IgE immunoglobulins are present. This symptom complex is commonly termed the Katayama syndrome. It is more common and more severe in visitors to endemic areas in whom it may persist for 3 months or more, occasionally resulting in death.

Prolonged febrile period with circulating immune complexes

Intestinal inflammation and encephalitis occur acutely

## Chronic Stage

Approximately one half of all deposited eggs reach the lumen of the bowel or bladder and are shed from the body. Those retained induce inflammation and scarring, initiating the final and most morbid phase of schistosomiasis. Soluble antigens excreted by the eggs stimulate the formation of T lymphocyte–mediated eosinophilic granulomas. Early in the infection, the inflammatory response is vigorous, producing lesions more than 100-fold larger than the inciting egg itself. Obstruction of blood flow is common. With time, the host's inflammatory response moderates, leading to a significant decrease in granuloma size. Fibroblasts stimulated by factors released by both retained eggs and the granulomas lay down scar tissue, rendering the earlier, granuloma-induced vascular obstruction permanent. As would be expected, the severity of tissue damage is directly related to the total number of eggs retained.

Inflammatory and fibrotic reactions to retained eggs cause chronic disease

In *S. haematobium* infection, the bladder mucosa becomes thickened, papillated, and ulcerated. Hematuria and dysuria result; repeated hemorrhages produce anemia. In severe infections the muscular layers of the bladder are involved, with loss of bladder capacity and contractibility. Vesicoureteral reflux, ureteral obstruction, and hydronephrosis may follow. Progressive obstruction leads to renal failure and uremia. Calcification of the bladder wall is occasionally seen, and approximately 10% of patients harbor urinary tract calculi. Secondary bacterial infections are common. Chronic *Salmonella* bacteriuria with recurrent bouts of bacteremia have been reported from Egypt. In the same country, bladder carcinoma is frequently seen as a late complication of disease.

*S. haematobium* produces bladder lesions with hemorrhage and obstruction

Chronic urinary carriage of *Salmonella* may cause bacteremia

In *S. mansoni* and *S. japonicum* infections, the bowel mucosa is congested, thickened, and ulcerated. Polyposis has been reported from Egypt but nowhere else. Patients experience abdominal pain, diarrhea, and blood in the stool. Eggs deposited in the larger intestinal veins may be carried by the portal blood flow back to the liver, where they lodge in the presinusoidal capillaries. The resulting inflammatory reaction leads to the development of periportal fibrosis and hepatic enlargement. The frequency and severity with which the liver is involved are genetically determined and associated with the human leukocyte antigen (HLA) type of the patient. In most cases, liver function is well preserved. Infected individuals who subsequently acquire hepatitis B or C viruses develop chronic active hepatitis more frequently than those free of schistosomes. The presinusoidal obstruction to blood flow can result in the serious manifestations of portal obstruction. Eggs carried around the liver in the portosystemic collateral vessels may lodge in the small pulmonary arterioles, where they produce interstitial scarring, pulmonary hypertension, and right ventricular failure. Immune complexes shunted to the systemic circulation may induce glomerulonephritis. Occasionally, eggs may be deposited in the central nervous system, where they may cause epilepsy or paraplegia.

Severity of liver involvement linked to HLA type

Hepatitis B or C superinfection may progress to chronic active hepatitis

Some differences between the clinical presentation of schistosomiasis mansoni and that of schistosomiasis japonicum have been noted. Manifestations of the latter disease typically occur earlier in the course of the infection and tend to be more severe. When involvement of the central nervous system develops, it is more likely to occur in the brain than the spinal cord. On the other hand, immune complex nephropathy and recurrent *Salmonella* bacteremia are more likely to be seen in hepatosplenic *S. mansoni* infections. The latter phenomenon is apparently related to the ability of *Salmonella* to parasitize the gut and integument of the adult fluke, providing a persistent bacterial focus within the portal system of the infected patient. This focus cannot be eradicated without treatment of the schistosomal infection.

Elimination of *Salmonella* focus requires eradication of parasite

## DIAGNOSIS

Definitive diagnosis requires the recovery of the characteristic eggs in urine, stool, or biopsy specimens. In *S. haematobium* infections, eggs are most numerous in urine samples obtained at midday. When examination of the sediment yields negative results, eggs may sometimes be recovered by filtering the urine through a membrane filter. Cystoscopy with biopsy of the bladder mucosa may be required for the diagnosis of mild infection. Eggs of *S. mansoni* and *S. japonicum* are passed in the stool. Concentration techniques such as formalin–ether or gravity sedimentation are necessary when the ova are scanty. Results of rectal biopsy may be positive when those of repeated stool examinations are negative.

*S. haematobium* eggs found in urine

*S. mansoni* and *S. japonicum* eggs in stool; rectal biopsy

Because dead eggs may persist in tissue for a long time after the death of the adult worms, active infection is confirmed only if the eggs are shown to be viable. This confirmation may be obtained by observing the eggs microscopically for movement of flame cell cilia or by hatching them in water. Quantitation of egg output is useful in estimating the severity of infection and in following response to treatment.

Determination of egg viability and output useful

Conventional serologic tests detect circulating antibodies with sensitivities exceeding 90% but cannot distinguish active from inactive infection. Recently introduced enzyme immunoassay (EIA)–based reagent strip (dipstick) tests capable of detecting circulating, genus-specific, adult-worm **antigens** in blood and urine are rapid, simple and sensitive. They are particularly helpful in the diagnosis of the Katayama syndrome in individuals returning from endemic areas. Moreover, because antigen levels drop rapidly after successful therapy, these tests may prove helpful in distinguishing active from inactive disease.

EIA detection of antigens in blood and urine

## TREATMENT

No specific therapy is available for the treatment of schistosomal dermatitis or the Katayama syndrome. Antihistamines and corticosteroids may be helpful in ameliorating their more severe manifestations. In the late stage of schistosomiasis, therapy is directed at interrupting egg deposition by killing or sterilizing the adult worms. Because the severity of clinical and pathologic manifestations is related to the intensity of infection, therapy of long-term residents of endemic areas is often reserved for patients with moderate or severe active infections.

Several anthelmintic agents may be used. Praziquantel, which is active against all three species of schistosomes, is the agent of choice. Unfortunately, several recent reports have suggested increased resistance to this single-dose oral agent in areas where it has been used in mass therapy programs. *S. mansoni* infections acquired in such areas may be treated with oxamniquine. Use of this agent is contraindicated in pregnancy.

Multiple anthelmintic drugs are used

## PREVENTION

It has proved both difficult and expensive to control this deadly disease. Programs aimed at interrupting transmission of the parasite by the provision of pure water supplies and the sanitary disposal of human feces are often beyond the economic reach of the nations most seriously affected. Similarly, measures to deny snails access to newly irrigated lands are expensive. Chemical molluscicides have been shown effective in limited trials, but have been less successful when used over large areas for prolonged periods. Mass therapy of the infected human population has, until recently, been severely limited by the toxicity of effective agents. Newer agents, particularly praziquantel, has proven more suitable for this purpose. Nevertheless, discontinuation of mass therapy, in the absence of other control measures, can result in a rapid rebound of active disease. At present, programs that have incorporated all of these control measures have been the most successful.

Sanitary disposal of feces often limited by economic status

Molluscicides effective but large-scale application difficult

Currently, there is intense interest in developing a vaccine suitable for human use. A vaccine made from irradiated *S. bovis* cercariae, developed for cattle, appears to confer a significant degree of protection against infection. Although the use of a similar live vaccine would not be suitable for human populations, the success of the animal vaccine has provided clues to potential immunoprotective mechanisms in human schistosomiasis. Monoclonal antibodies have been used to identify a number of schistosomula and adult antigens thought to be capable of inducing protective immunity; the World Health Organization has selected six of these for further evaluation.

Vaccines under development

## ADDITIONAL READING

Bergquist NR, Colley DG. Schistosomiasis vaccines. *Parasitol Today* 1998;14:99–104. Several companion articles in the same publication explore the current status of schistosomiasis vaccine development.

Capron A. Schistosomiasis: forty years' war on the worm. *Parasitol Today* 1998;14: 379–384. This is the opening article in a journal issue devoted exclusively to the control of schistosomiasis.

Hagen P. Reinfection, exposure and immunity in human schistosomiasis. *Parasitol Today* 1992;8:12–16. A brief, clear summary of a very complex topic.

Montenegro SML, et al. Cytokine production in acute versus chronic schistosomiasis: The cross-regulatory role of interferon-$\gamma$ and interleukin-10 in the responses of peripheral blood mononuclear cells and splenocytes to parasite antigens. *J Infect Dis* 1999;179:1502.

Mostafa MH, Sheweita SA, O'Connor PJ. Relationship between schistosomiasis and bladder cancer. *Clin Microbiol Rev* 1999;12:97–111.

Yee B, et al. Pulmonary paragonimiasis in Southeastern Asians live in the Central San Joaquin valley. *West J Med* 1992;156:423–425.

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# PART IX

# LOCAL AND SYSTEMIC INFECTIONS

## CHAPTER 64

Middle and Lower Respiratory Tract Infections

## CHAPTER 65

Enteric Infections and Food Poisoning

## CHAPTER 66

Urinary Tract Infections

## CHAPTER 67

Central Nervous System Infections

## CHAPTER 68

Intravascular Infections, Bacteremia, and Endotoxemia

## CHAPTER 69

Infections of the Fetus and Newborn

## CHAPTER 70

Sexually Transmitted Diseases

## CHAPTER 71

Infections in the Immunocompromised Patient

## CHAPTER 72

Nosocomial Infections and Infection Control

CHAPTER 59

# Skin and Wound Infections

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## SKIN INFECTIONS

Infections of the skin can result from microbial invasion from an external source or from organisms reaching the skin through the bloodstream as part of a systemic disease. Blood-borne involvement is evidenced by rashes in many viral and bacterial infections, such as measles and secondary syphilis, or may yield more chronic granulomatous skin lesions in blastomycosis, tuberculosis, and syphilis. Skin lesions remote from sites of infection can be produced by some bacterial toxins, such as the pyrogenic exotoxins of group A streptococci and *Staphylococcus aureus.* They can also result from immunologic responses to microbial antigens that have reached the skin. Thus, there are manifold skin manifestations of infections; however, this chapter will be restricted to the discussion of direct infections that may occur in the Western Hemisphere.

Skin lesions may be primary or the result of bacteremia

The skin is an organ system with multiple functions, including protection of the tissues from external microbial invasion. Its keratinized stratified epithelium prevents direct microbial invasion under normal conditions of surface temperature and humidity, and its normal flora, pH, and chemical defenses tend to inhibit colonization by many pathogens (see Chapters 9 and 10). However, the skin is subject to repeated minor traumas that are often unnoticed but that destroy its integrity and allow organisms to gain access to its deeper layers from the external environment. The surface is also penetrated by ducts of pilosebaceous units and sweat glands, and microbial invasion can occur along these routes, particularly if the ducts are obstructed.

Trauma and the appendages of the skin provide access

### Infections in Hair Follicles, Sebaceous Glands, and Sweat Glands

#### *Folliculitis*

Folliculitis is a minor infection of the hair follicles and is usually caused by *S. aureus*. It is often associated with areas of friction and of sweat gland activity and is thus seen most frequently on the neck, face, axillae, and buttocks. Blockage of ducts with inspissated sebum, as in acne vulgaris, predisposes to the condition. Folliculitis can also be caused by *Pseudomonas aeruginosa,* and this form of the disease has become more common in recent years, with the popularity of hot tubs and whirlpool baths. Unless these facilities are thoroughly cleansed and adequately chlorinated, they can grow large numbers of pseudomonads at their normal operating temperatures, causing extensive folliculitis on areas of the body that have been immersed. The lesions subside rapidly when the insult is

Staphylococci and *Pseudomonas* infect hair follicles discontinued. Occasionally, folliculitis may be caused by infection with *Candida albicans*. Such cases are particularly common in immunocompromised hosts.

*Propionibacterium acnes* contributes to inflammation of acne

**Acne vulgaris** also involves inflammation of hair follicles and associated sebaceous glands. The comedo of acne results from multiplication of *Propionibacterium acnes,* the predominant anaerobe of the normal skin, behind and within inspissated sebum. Organic acids produced by the organism are believed to stimulate an inflammatory response and thus contribute to the disease process. However, the primary cause of the disease is hormonal influences on sebum secretion that occur at puberty, and the disease usually resolves in early adult life.

### *Furuncles*

Staphylococcal furuncles are skin abscesses that can spread

The furuncle is a small staphylococcal abscess that develops in the region of a hair follicle. Furuncles may be solitary or multiple and may constitute a troublesome recurrent disease. Spread of infection to the dermis and subcutaneous tissues can result in a more extensive multiloculated abscess, the **carbuncle.** These lesions and their treatment are considered in Chapter 16.

### *Treatment*

Skin care and tetracycline may be used

Folliculitis and individual furuncles are normally treated locally by measures designed to establish drainage without the use of antibiotics. Chronic furunculosis may require attempts to eliminate nasal carriage of *S. aureus,* which is sometimes the source of the infection. Antimicrobics are not usually required unless surrounding cellulitis or carbuncles develops. Severe acne can often be treated effectively with topical drying agents. Prolonged administration of low oral doses of a tetracycline or macrolide is often effective, although the reason for the therapeutic response is uncertain.

## Infections of Other Skin Layers

Minor or inapparent skin lesions serve as the route of infection in many localized skin infections and in some systemic diseases, such as syphilis and leptospirosis.

### *Infection of Keratinized Layers*

Inflammatory response is important with dermatophytes

Cell-mediated immunity defects in chronic candidiasis

The only organisms that can use the keratin on cells, hairs, and nails are the dermatophyte fungi. The dermatophytes are particularly well adapted to these sites, cannot grow at 37°C, and fail to invade deeper layers. The clinical manifestations of these infections result from the inflammatory and delayed hypersensitivity responses of the host, and the desquamation induced by these processes is a major factor in the ultimate control of the infection by removing infected skin. In candidiasis, control involves cell-mediated immune mechanisms, and chronic *Candida* skin and nail infections are often associated with defects in cellular immunity.

### *Impetigo*

Group A streptococci are primary cause

*S. aureus* may colonize or act as primary pathogen

Pyoderma, also termed impetigo, is a common, sometimes epidemic skin lesion. This disease is caused primarily by group A streptococci. The initial lesion is often a small vesicle that develops at the site of invasion and ruptures with superficial spread characterized by skin erosion and a serous exudate, which dries to produce a honey-colored crust. The exudate and crust contain numerous infecting streptococci. *S. aureus* may occasionally produce pustular impetigo or contaminate the lesions caused by streptococci. Epidemic impetigo is most common in childhood and under conditions of heat, humidity, poor hygiene, and overcrowding. The infection may be spread by fomites such as shared clothing and towels. It is sometimes caused by nephritogenic strains of *S. pyogenes,* particularly in the tropics, and acute glomerulonephritis may result. Rheumatic fever is not associated with streptococcal lesions of the skin.

Treatment is usually with penicillin or erythromycin and topical antimicrobics or skin antiseptics to limit spread.

Bullous impetigo is a distinct disease caused by strains of *S. aureus* that produce exfoliation. It is most common in small children, but may occur at any age. The infection is characterized by large serum-filled bullae (blisters) within the skin layers at the site of infection. Minor infections are treated topically; however, bullous impetigo in infants is a serious disease that usually requires systemic antimicrobic treatment. Epidemic spread may occur under conditions similar to those described for streptococcal impetigo.

Bullous impetigo is caused by exfoliation-producing *S. aureus*

### *Erysipelas*

Erysipelas is a rapidly spreading infection of the deeper layers of the dermis that is almost always caused by group A streptococci. It is associated with edema of the skin; marked erythema; pain; and systemic manifestations of infection, including fever and lymphadenopathy. Because the infection is intradermal, the streptococci cannot usually be isolated from the skin surfaces. The disease can progress to septicemia or local necrosis of skin. It is serious and requires immediate treatment with penicillin or erythromycin.

Group A streptococcal erysipelas is a spreading cellulitis with risk of bacteremia

### *Cellulitis*

Cellulitis is not a skin infection as such, but it can develop by extension from skin or wound infections. It usually presents as an acute inflammation of subcutaneous connective tissue with swelling and pain and often with marked constitutional signs and symptoms. It can be caused by many pathogenic bacteria, but *S. aureus* and group A streptococci are most common. *Haemophilus influenzae* type b is a cause in infants and children. Enteric Gram-negative rods, clostridia, and other anaerobes may also cause cellulitis as a complication of wound infections, particularly in immunocompromised hosts and individuals with uncontrolled diabetes.

Most often caused by pyogenic cocci or *H. influenzae* in children

## Skin Ulcers and Granulomatous Lesions

Many acute and subacute skin infections are characterized by ulceration or a granulomatous response. Some are sexually transmitted and are discussed in Chapter 70. Others derive from systemic infection and are not direct infections of skin. A few examples of direct infections, which pose special diagnostic problems, are considered below. Herpes simplex virus can invade through the skin to produce a local vesicular lesion followed by ulceration. The lesion may then recur in the infected area. Primary herpetic lesions of the finger can mimic staphylococcal paronychia very closely, as well as produce lymphangitis and local lymph node enlargement with pain and fever.

Herpetic paronychia can mimic staphylococcal infections

Skin diphtheria, which remains common in some tropical areas, also occurred endemically among the transient population of the West Coast of the United States during the 1970s and early 1980s. The organism gains access through a wound or insect bite and causes chronic erosion and ulceration of the skin, sometimes with evidence of the systemic effects of diphtheria toxin.

Skin diphtheria is seen in transients

*Mycobacterium marinum* produces a self-limiting granuloma, usually of the forearms and knees. The organism usually enters through superficial abrasions from rocks or swimming pool walls. Infections with *M. ulcerans* are more serious and produce progressive ulceration, but are limited to tropical areas and do not occur in the United States or Europe. Several rare forms of necrotic spreading skin ulceration tend to develop in immunosuppressed hosts, in diabetics, and as complications of abdominal surgery. These lesions include bacterial synergistic gangrene, caused by mixtures of peptostreptococcus, *S. aureus,* and group A streptococci. Variants of these conditions produce extensive and spreading necrotic cellulitis. The major form of treatment is to excise the infected tissues widely and supplement such surgery with massive chemotherapy.

Mycobacterial species cause granulomas

Synergistic gangrene may require surgery

Several primary fungal diseases are associated with cutaneous ulceration or cellulitis, including mycetoma and chromoblastomycosis, which involve the feet, and sporotrichosis, in which ulceration often develops from infected subcutaneous lymph nodes and

Fungal and parasitic ulcerations are usually related to trauma vessels. Likewise, some parasites directly infect and ulcerate the skin, as in cutaneous leishmaniasis and cutaneous amebiasis. These latter two diseases are not contracted in the United States.

## WOUND INFECTIONS

Wounds subject to infection can be surgical, traumatic, or physiologic. The latter include the endometrial surface, after separation of the placenta, and the umbilical stump in the neonate. Traumatic wounds comprise such diverse damage as deep cuts, compound fractures, frostbite necrosis, and thermal burns. Sources of infection include (1) the patient's own normal flora; (2) material from infected individuals or carriers that may reach the wound on fomites, hands, or through the air; and (3) pathogens from the environment that can contaminate the wound through soil, clothing, and other foreign material. Examples of such infections include contamination of a penetrating stab wound to the abdomen by colonic flora, contamination of a clean surgical wound in the operating room with *S. aureus* spread from the flora of a perineal carrier, and introduction of spores of *Clostridium tetani* into the tissues on a splinter.

Sources of infection include patient, environment, and infected persons

### Classification of Wounds

Surgical and traumatic wounds are classified according to the extent of potential contamination and thus, the risk of infection. These criteria carry important implications regarding surgical treatment and chemoprophylaxis. **Clean wounds** are surgical wounds made under aseptic conditions that do not traverse infected tissues or extend into sites with a normal flora. **Clean contaminated wounds** are operative wounds that extend into sites with a normal flora (except the colon) without known contamination. **Contaminated wounds** include fresh surgical and traumatic wounds with a major risk of contamination, such as incisions entering nonpurulent infected tissues. **Dirty and infected wounds** include old, infected traumatic wounds; wounds substantially contaminated with foreign material; and wounds contaminated with spillage from perforated viscera.

Wounds vary in risk of bacterial exposure

Infection rates in clean surgical wounds should be less than 1%, whereas untreated dirty wounds have a higher probability of infection. Similar considerations apply to the chance of infection developing in a placental site or on the umbilicus. A normal delivery without retained products will rarely be followed by endometrial infection. A prolonged delivery after rupture of the membranes with retained placental fragments poses an increased risk. In some rural cultures in Africa, soil is applied to the umbilical stump, and neonatal tetanus is common, whereas it is almost unknown in other cultures.

### Factors Contributing to Wound Infection

Various factors, in addition to those indicated previously, contribute to the probability of a wound becoming infected. The contaminating dose of microorganisms and their virulence can be critical and, other things being equal, the chance of infection developing increases progressively with the contaminating dose. The physical and physiologic condition of the wound also influences the probability of infection. Areas of necrosis, vascular strangulation from excessively tight sutures, hematomas, excessive edema, poor blood supply, and poor oxygenation all compromise normal defense mechanisms and substantially reduce the dose of organisms needed to initiate infection. Thus, removal of necrotic tissue and the surgeon's skill, gentleness, and attention to detail are major factors in preventing the development of infection.

Infectious risk increases with contaminating dose of organisms

Vascular integrity is important for defense

The general health, nutritional status, and ability of patients to mount an inflammatory response are also major determinants of whether a wound infection develops. Infection rates are higher in the elderly, the obese, individuals with uncontrolled diabetes, and those on immunosuppressive or corticosteroid therapy. Nutritional deficiencies enhance the risk of infection, and new approaches to avoid protein–calorie malnutrition in patients with severe burns, for example, have led to substantial reductions in serious clinical infections.

Nutritional and immunologic status and inflammatory response of the host

There is strong evidence that the critical period determining whether contamination of surgical wounds proceeds to infection lies within the first 3 hours after contamination.

For this reason, prophylactic chemotherapy of some surgical wounds and procedures can be restricted to the operative and immediate perioperative period. There is general agreement that extending such prophylaxis beyond 24 hours increases the chance of complications without reducing the risk of infection.

First 3 hours is critical period for surgical wounds

## Treatment and Prevention

Severe wound infections are almost always treated with a combination of surgical and chemotherapeutic approaches. Necrotic tissue and contaminated foreign bodies, such as sutures, must be removed, pockets of pus opened, and drainage established. This approach permits access of the appropriate antibiotics to viable tissues in which they can act. Epidemiologic approaches to the prevention of wound infection and the appropriate uses of chemoprophylaxis are considered in Chapter 13. There has been increasing interest in the possibilities of active or passive immunization against common Gram-negative antigens. Despite some encouraging experimental results, the clinical application of these findings to burns and severe trauma seems distant.

Chemoprophylaxis is mainstay

Immunization is not practical

# ETIOLOGIC AGENTS

Some major causes of skin and wound infections are shown in Table 59–1. *S. aureus* remains the single most common cause of infection of clean surgical wounds; however, the number of infections caused by opportunistic Gram-negative organisms is increasing. This finding reflects the extension of surgical intervention to more patients whose defenses are compromised or who would have been unacceptable surgical risks before the introduction of new technical and therapeutic procedures. Severe invasive group A streptococcal infections with the toxic shock-like syndrome often begin with a simple skin or wound infection.

*S. aureus* and Gram-negative bacteria are most common

Streptococcal toxic shock begins with wound

Anaerobic Gram-negative wound infections have been reported increasingly in the last two decades or so as a result of the higher incidence of such infections in immunocompromised patients and better laboratory recognition. Most infecting organisms derive from normal floral sites and the majority are *Bacteroides,* often in combination with anaerobic Gram-positive cocci and facultative aerobic bacteria. They tend to be associated with necrosis, which may spread subcutaneously, and with thrombophlebitis, which may lead to bacteremia. Most postpartum uterine infections are now caused by Gram-negative anaerobes or anaerobic Gram-positive cocci; they can range from self-limiting infections to severe infections of the uterus with pelvic thrombophlebitis. Human bite wounds are particularly subject to anaerobic infections. In contrast, infected bites of domestic animals (dogs, cats) are almost always due to *Pasteurella multocida*.

*Bacteroides* and anaerobic Gram-positive coccal infections derived from patient's flora

Burns and areas of necrosis resulting from vascular stasis or insufficiency are subject to infection with the same organisms that predominate in postsurgical wound infections. However, *P. aeruginosa* causes particularly serious infections in burns, with loss of skin grafts and a high risk of septicemia and death. If the fluid electrolyte and nutritional deficiencies of a burned patient can be controlled, the greatest hazard to life is infection.

*P. aeruginosa* is a virulent cause of burn infections

Tetanus remains a threat to the unimmunized or inadequately immunized individual, particularly from heavy contamination of puncture wounds or introduction of foreign bodies such as splinters, soil, or clothing into the subcutaneous tissues. *C. tetani* never spreads beyond the site of the local lesion, and adequate circulating antibody from tetanus toxoid immunization will prevent the development of the disease. Gas gangrene (clostridial myositis) can develop within a few hours of traumatic injury and lead to rapid death. *C. perfringens* is the most common cause, and its $\alpha$-toxin produces the spreading tissue damage and muscle death. Other aerobic and anaerobic bacteria are invariably present and sometimes play an important etiologic role. The disease is always associated with muscle trauma and necrosis, which provide the conditions for anaerobic multiplication. Compound fractures, gunshot wounds, and similar extensive injuries that allow entry of clostridial spores set the stage for the disease. Prevention involves surgically debriding all necrotic or potentially necrotic tissue as soon as possible and administering high-dose penicillin.

Tetanus is derived from the environment

Gas gangrene requires surgical intervention

TABLE 59–1

**Major Causes of Skin and Wound Infections**

| Syndrome | Bacteria | Fungi | Other |
|---|---|---|---|
| Impetigo | Group A streptococci<br>*Staphylococcus aureus* | | |
| Folliculitis | *Pseudomonas aeruginosa*<br>*Staphylococcus aureus* | *Candida albicans* | |
| Acne | *Propionibacterium acne* | | |
| Furuncle | *Staphylococcus aureus* | | |
| Cellulitis | Group A streptococci[a]<br>*Staphylococcus aureus*<br>*Haemophilus influenzae* | | |
| Intertrigo | *Staphylococcus aureus*<br>Enterobacteriaceae | *Candida albicans* | |
| Chronic ulcers[b] | *Treponema pallidum*<br>*Haemophilus ducreyi*<br>*Corynebacterium diphtheriae*<br>*Bacillus anthracis*<br>*Nocardia*<br>*Mycobacterium* | *Sporothrix* | Herpesvirus |
| Wounds | | | |
| Trauma | *Clostridium*<br>Enterobacteriaceae<br>*Pseudomonas aeruginosa* | | |
| Surgical (clean) | *Staphylococcus aureus*<br>Enterobacteriaceae<br>Group A streptococci | | |
| Surgical (dirty)[c] | *Staphylococcus aureus*<br>Enterobacteriaceae<br>Anaerobes | | |
| Burns | *Pseudomonas aeruginosa*<br>*Staphylococcus aureus*<br>Enterobacteriaceae | *Candida albicans* | |
| Animal bites | *Pasteurella multocida* | | |

[a] Including "erysipelas," an infection primarily involving the deeper layers of the dermis.

[b] Usually begin as nodules or pustules.

[c] Etiology determined by the origin of the contaminating flora (eg, abdominal vs. gynecologic surgery).

## ADDITIONAL READING

Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* 2001;14:244–269. This review emphasizes the surgical side of wound management and experimental study.

CHAPTER 60

# Bone and Joint Infections

C. George Ray

Infections of bones and joints may exist separately or together. Both are most common in infancy and childhood. They are usually caused by blood-borne (hematogenous) spread to the infected site but can also result from local trauma with secondary infection. Sometimes there may be local spread from a contiguous soft tissue infection, often associated with the presence of a foreign body at the site of the primary wound.

The local effect of such infections can be devastating if they are inadequately treated, because inflammation and resultant tissue necrosis may produce irreparable damage. The presence of pus under pressure can compromise normal blood flow and even cause destruction of blood vessels with avascular necrosis of tissue. When this condition develops, a **sequestrum** can result, in which a part of the cartilage or bone becomes totally separated from its blood supply and cannot be incorporated into the healing process. In some patients, sequestrum formation can lead to a smoldering chronic infection with draining sinuses and loss of functional integrity. Normal growth of the affected site can be severely impaired in the infant or child, particularly when the epiphysis is involved. In the acute phase of infection, bacteremia may also cause sepsis and metastatic infections in sites such as the lungs and heart. The result may be fatal.

Sequestrum formation can lead to chronic infection with draining sinuses

Infection can cause growth impairment in children

Bacteremia and metastatic spread from bone and joint infections is common

## OSTEOMYELITIS

The onset of acute hematogenous osteomyelitis is usually abrupt but can sometimes be quite insidious. It is classically characterized by localized pain, fever, and tenderness to palpation over the affected site. More than one bone or joint may be involved as a result of hematogenous spread to multiple sites. With progression, the classic signs of heat, redness, and swelling may develop. Laboratory findings often include leukocytosis and elevated acute-phase reactants, such as C-reactive protein and sedimentation rate. Osteomyelitis caused by a contiguous focus of infection is usually associated with the presence of local findings of soft tissue infection, such as skin abscesses and infected wounds.

Local pain and signs of inflammation

When osteomyelitis occurs in close proximity to a joint, septic arthritis may develop by direct spread through the epiphysis (usually in infants) or by lateral extension through the periosteum into the joint capsule. Such extension is particularly common in hip and elbow infections.

May come from contiguous focus

Extend to joints through epiphysis and adjacent periosteum

### Common Etiologic Agents

The most common causes of acute osteomyelitis and those associated with special circumstances are shown in Table 60–1. It is clear that age plays a significant role in

TABLE 60–1

**Common Causes of Acute Osteomyelitis**

| Situation | Usual Causative Organism |
|---|---|
| **Age group** | |
| Neonates (<1 mo) | *Staphylococcus aureus,* group B streptococci, Gram-negative rods (eg, *Escherichia coli, Klebsiella, Proteus, Pseudomonas*) |
| Older infants, children, adults | *S. aureus, S. pneumoniae, Kingella kingae* |
| **Special problems** | |
| Chronic hemolytic disorders (eg, sickle cell disease) | *S. aureus, S. pneumoniae, Salmonella* species |
| Infection after trauma or surgery | *S. aureus,* group A streptococci, Gram-negative aerobic or anaerobic bacteria |
| Infection after puncture wound of foot | *Pseudomonas aeruginosa, S. aureus* |

Age-related etiologies, but staphylococcal osteomyelitis most common

Chronic granulomatous osteomyelitis suggests mycobacteria or fungi

influencing the relative frequency of the various infective agents, particularly in early infancy; however, most infections are caused by *Staphylococcus aureus.*

Low-grade smoldering infections may also occur with the organisms listed in Table 60–1; however, chronic granulomatous processes must also be considered, including tuberculosis, coccidioidomycosis, histoplasmosis, and blastomycosis. These latter infections usually result from systemic dissemination, and the lesions develop slowly over a period of months. Occasionally bone tumors or cysts and leukemia must also be considered in the differential diagnosis.

## Diagnostic Approaches

The primary goals of diagnosis are to establish the existence of infection and to determine its cause. The following procedures are generally used:

Blood cultures, direct aspirates, and bone scans

X-rays may be normal in early stages of infection

1. Blood cultures, because many infections are associated with bacteremia.
2. Radionuclide scanning or magnetic resonance imaging to demonstrate evidence of localized infection.
3. Direct staining, culture, and histology of needle aspirates or biopsies of periosteum or bone.
4. X-rays of affected sites, which often appear normal in the early stages of infection. The first changes seen are swelling of surrounding soft tissues, followed by periosteal elevation. Demineralization of bone may not become apparent for 2 weeks or more after the onset of symptoms; calcification of the periosteum and surrounding soft tissues is usually delayed even longer.

## Management Principles

Bactericidal antimicrobics continued for weeks

Surgery and prolonged therapy required for chronic osteomyelitis

In acute infections, early intervention is important. Management includes vigorous use of bactericidal antimicrobics, which must often be continued for several weeks to ensure a bacteriologic cure and prevent progression to chronic osteomyelitis. Surgical drainage is also essential if there is significant pressure from the localized, purulent process. In chronic osteomyelitis, sequestrum formation is frequent and sinuses may develop that drain the bone abscess to the skin surface. The infection is persistent, and treatment becomes extremely difficult. Such patients often require long-term antibiotic treatment (months to years) combined with surgical procedures to drain the abscesses and remove necrotic, infected tissues in an attempt to control infection while preserving the integrity of the affected bone.

## SEPTIC ARTHRITIS

The usual clinical features of septic arthritis include onset of pain, which is often abrupt and accompanied by fever. Single or multiple joints may be involved. Tenderness and swelling of the affected joints and frequently other signs of local inflammation are present. Attempts to move the joints, either actively or passively, result in severe pain. In infants, the symptoms may be somewhat nonspecific; local swelling or excessive irritability with unwillingness to move the affected extremity (pseudoparalysis) may be the only clues to the diagnosis.

Pain on movement with swelling and fever

### Common Etiologic Agents

The major causes of septic arthritis are listed in Table 60–2. Although *S. aureus* infection can occur at any age, there are some significant age-specific relationships to other bacterial causes. There is a high frequency of group B streptococcal infections in neonates, whereas in children between 1 month and 4 years of age, pneumococci are more likely to be involved. *Haemophilus influenzae* type b disease, which was once quite common in this age group, has been markedly diminished in the last decade; this is believed to be due to widespread use of an effective vaccine. *Neisseria gonorrhoeae* is implicated in most cases of septic arthritis in young adults. Subacute or chronic infective arthritis should prompt consideration of tuberculosis, Lyme disease, syphilis, and fungal infections such as coccidioidomycosis or *Candida.* Arthritis attributable to *Candida* is particularly likely in immunocompromised patients.

*S. aureus* appears at any age

Other pyogenic cocci are related to age and behavior

Tuberculous, spirochetal, and fungal arthritis have subacute or chronic course

Viruses and *Mycoplasma* can also cause acute arthritis in single or multiple joints. Such illnesses have been associated with rubella, hepatitis B, mumps, parvovirus B19, varicella, Epstein–Barr virus, coxsackievirus, and adenovirus infections, as well as with *M. pneumoniae* and *M. hominis.* These arthritides are usually self-limiting and rarely require specific therapy. Some bacterial infections of sites other than joints may be associated with noninfectious (reactive) arthritis, possibly resulting from deposition of circulating immune complexes and complement in synovial tissues, leading to inflammation. This has occurred with intestinal infections caused by *Yersinia enterocolitica, Campylobacter jejuni,* and some *Salmonella* species and also as a delayed sequela after successful treatment of sepsis due to *N. meningitidis* or *H. influenzae.*

Viral or *Mycoplasma* arthritis is usually self-limiting

Immune complexes from other sites may cause reactive arthritis

Noninfectious causes of arthritis must also be considered in the differential diagnosis. They can closely mimic septic arthritis. Examples include inflammatory collagen vascular disease such as rheumatoid arthritis, gout, traumatic arthritis, and degenerative arthritis.

TABLE 60–2

**Common Causes of Septic Arthritis**

| Age Group | Usual Causative Organism |
|---|---|
| Neonate (<1 mo) | *Staphylococcus aureus,* group B streptococci, Gram-negative rods (eg, *Escherichia coli, Klebsiella, Proteus, Pseudomonas*) |
| 1 mo–4 yr | *S. aureus,* group A streptococci, *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae* type b |
| 4–16 yr | *S. aureus, Streptococcus pyogenes* |
| 16–40 yr | *Neisseria gonorrhoeae, S. aureus* |
| >40 yr | *S. aureus* |

TABLE 60–3

**Findings in Synovial Fluid in Various Forms of Arthritis**

| Laboratory Test | Normal | Septic Bacterial Arthritis | Trauma, Degenerative Joint Disease | Rheumatoid Arthritis, Gout |
|---|---|---|---|---|
| Clarity and color | Clear | Opaque, yellow to green | Clear, yellow | Translucent, yellow; or opalescent |
| Viscosity | High | Variable | High | Low |
| White blood cells/$mm^3$ | <200 | 25,000–100,000 | 200–2000 | 2000–20,000 |
| Polymorphonuclear cells (%) | <25 | >75 | 25–50 | ≥50 |
| Glucose level (relative to simultaneous blood glucose level) | Nearly equal | <25% | Nearly equal | 50–80% |

## Diagnostic Approaches

In acute cases, blood cultures are often useful because bacteremia may be present. The definitive diagnosis is established by examination of synovial fluid removed from the joint by needle aspiration (arthrocentesis). Because other noninfectious causes must be considered, it is important to analyze the chemical and cellular characteristics of the fluid in addition to performing a Gram stain and culture. Table 60–3 summarizes the major findings in synovial fluid in normal and various disease states. Septic bacterial arthritis is usually associated with grossly purulent fluid containing more than 25,000 white blood cells per cubic millimeter, predominantly polymorphonuclear cells. The glucose level in the synovial fluid is usually less than 25% of that in the blood.

Blood culture is particularly useful

Needle aspiration of synovial fluid is used for analysis and culture

In viral, tuberculous, and fungal arthritis, as well as in partially treated bacterial arthritis, cell counts are usually lower, and mononuclear cells may constitute a greater proportion of the inflammatory cells. Occasionally, biopsy of the synovial membrane may be required to resolve the diagnosis. Histologic examination and culture of the tissue are particularly helpful in distinguishing granulomatous from rheumatoid disease.

Biopsy is especially useful in chronic cases

In most cases of acute septic arthritis, the blood culture and/or synovial fluid culture yields the specific etiologic agent. One major exception is *N. gonorrhoeae,* which can be difficult to isolate from these sources. When this organism is suspected, it is wise to include cultures of other sites of potential infection, such as the urethra, cervix, rectum, and pharynx, as well as skin lesions.

Gonococci may be difficult to isolate from joint fluid

## Management Principles

Prompt, vigorous, systemic antimicrobial therapy is required as soon as diagnostic tests suggest a bacterial cause. This treatment usually must be continued for 3 to 6 weeks, depending on the etiologic agent and the clinical response to therapy. Drainage of pus under pressure is also an important aspect of management. In cases of hip joint involvement, open surgical drainage is often necessary because collateral blood supply to the hip joint is relatively limited, and pus under pressure can lead to irreversible avascular necrosis of the tissues with permanent crippling. It is also difficult to evaluate the amount of pus that may be present because of the overlying muscles. Other joints can usually be managed by simple aspiration of pus whenever it reaccumulates significantly during the acute phase of infection.

Drainage of hip infections often necessary

CHAPTER 61

# Eye, Ear, and Sinus Infections

C. George Ray

## Eye Infections

Ocular infections can be divided into those that primarily involve the external structures—eyelids, conjunctiva, sclera, and cornea—and those that involve internal sites. The major defense mechanisms of the eye are the tears and the conjunctiva, as well as the mechanical cleansing that occurs with blinking of the eyelids. The tears contain secretory IgA and lysozyme, and the conjunctiva possesses numerous lymphocytes, plasma cells, neutrophils, and mast cells, which can respond quickly to infection by inflammation and production of antibody and cytokines. The internal eye is protected from external invasion primarily by the physical barrier imposed by the sclera and cornea. If these are breached (eg, by a penetrating injury or ulceration), infection becomes a possibility. In addition, infection may reach the internal eye via the blood-borne route to the retinal arteries and produce chorioretinitis and/or uveitis. Such infections are a particularly common problem in immunocompromised patients.

Defenses of the eye include tears, conjunctiva, and blinking

Tears have sIgA and lysozymes

Other causes of inflammation of the external or internal eye can involve autoimmune or allergic mechanisms, which may be provoked by infectious agents or diseases such as rheumatoid arthritis.

Autoimmune and allergic causes of inflammation

### COMMON CLINICAL CONDITIONS

**Blepharitis** is an acute or chronic inflammatory disease of the eyelid margin. It can take the form of a localized inflammation in the external margin (hordeolum or stye) or a granulomatous reaction to infection and plugging of a sebaceous gland of the eyelid (chalazion).

**Dacryocystitis** is an inflammation of the lacrimal sac. It usually results from partial or complete obstruction within the sac or nasolacrimal duct, where bacteria may be trapped and initiate either an acute or a chronic infection.

**Conjunctivitis** is a term used to describe inflammation of the conjunctiva; it may extend to involve the eyelids, cornea (keratitis), or sclera (episcleritis). Extensive disease involving the conjunctiva and cornea is often called keratoconjunctivitis. Progressive keratitis can lead to ulceration, scarring, and blindness. **Ophthalmia neonatorum** is an acute, sometimes severe, conjunctivitis or keratoconjunctivitis of newborn infants.

**Endophthalmitis** is rare, but often leads to blindness even when treated aggressively. The term refers to infection of the aqueous or vitreous humor, usually by bacteria or fungi.

**Uveitis** consists of inflammation of the uveal tract—iris, ciliary body, and choroid. Although most inflammations of the iris and ciliary body (iridocyclitis) are not of infectious origin, some agents have been implicated. The acute disease may be associated with severe eye pain, redness, and photophobia; other cases may progress quite silently, with decreased visual acuity as the only symptom in the late stages. The most common infective involvement of the uveal tract is **chorioretinitis,** in which inflammatory infiltrates are seen in the retina; this infection can lead to destruction of the choroid and inflammation of the optic nerve (optic neuritis) and may extend into the vitreous humor to cause endophthalmitis. If the disease is not treated adequately, the end result can be blindness.

## COMMON ETIOLOGIC AGENTS

Blepharitis often staphylococcal

Acute conjunctivitis: age-related etiologies

Chronic conjunctivitis: *C. trachomatis* and herpes simplex

The major infectious causes of various inflammatory diseases of the eye are listed in Table 61–1. *Staphylococcus aureus* is the principal offender in bacterial infections of the eyelid and cornea. *Haemophilus influenzae* and *Streptococcus pneumoniae* are common causes of acute bacterial conjunctivitis. In young infants, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are significant causes of external eye disease, contracted from the mother's birth canal, that must be diagnosed and treated promptly. Chronic conjunctivitis or keratoconjunctivitis at any age must also prompt consideration of *C. trachomatis* infection. Herpes simplex is also a major cause of chronic or recurrent conjunctivitis, especially in infections of the external structures, and specific therapy is available. Epidemic

TABLE 61–1

**Major Infectious Causes of Eye Disease**

| Disease | Bacteria | Viruses | Fungi | Parasites |
|---|---|---|---|---|
| Blepharitis | *Staphylococcus aureus* | | | |
| Dacryocystitis | *Streptococcus pneumoniae, S. aureus* | | | |
| Conjunctivitis, keratitis, keratoconjunctivitis | *S. pneumoniae, Haemophilus influenzae, Haemophilus aegyptius, Streptococcus pyogenes, S. aureus, Chlamydia trachomatis, Neisseria gonorrhoeae, Neisseria meningitidis* | Adenoviruses, herpes simplex; measles, varicella–zoster | *Eusarium* species, *Aspergillus* species | *Acanthamoeba* (keratitis) |
| Ophthalmia neonatorum | *N. gonorrhoeae, Chlamydia trachomatis* | Herpes simplex | | |
| Endophthalmitis | *S. aureus, Pseudomonas aeruginosa,* other Gram-negative organisms | | *Candida* species, *Aspergillus* species | |
| Iridocyclitis | *Treponema pallidum* | Herpes simplex, varicella–zoster | | |
| Chorioretinitis | *Mycobacterium tuberculosis* | Cytomegalovirus, herpes simplex, varicella–zoster | *Histoplasma capsulatum, Coccidioides immitis, Candida* species | *Toxoplasma gondii, Toxocara canis* |

conjunctivitis or keratoconjunctivitis is most commonly associated with a variety of adenovirus serotypes. Outbreaks have been associated with inadequately chlorinated swimming pools, contaminated equipment or eyedrops in physicians' offices, and communal sharing of towels, which facilitates direct transmission.

Epidemic adenovirus conjunctivitis related to swimming pools and eyedrops

Chorioretinitis is frequently a manifestation of systemic disease (eg, histoplasmosis, tuberculosis) and congenital infections. It is particularly common in immunocompromised patients, who are liable to develop disseminated *Candida,* cytomegalovirus, or *Toxoplasma gondii* infections. Endophthalmitis may also result from blood-borne dissemination or by contiguous spread as a result of injury (eg, corneal ulcerations). In the latter situation, iatrogenic infection by agents such as *Pseudomonas* species can be induced by contaminated eye drops and ophthalmologic examination equipment.

Chorioretinitis usually linked to systemic disease, congenital infections, or immunocompromise

Endophthalmitis is from blood-borne or contiguous spread

Infection of the soft tissues surrounding the eye (periorbital or orbital cellulitis) is potentially severe and can spread to involve the functions of the eye itself. Major causes are *S. aureus, H. influenzae, Streptococcus pyogenes,* and *S. pneumoniae*.

## DIAGNOSTIC APPROACHES

In external bacterial infections of the eye, etiologic diagnoses can usually be established by Gram stain and culture of surface material or, in the case of viral infections, by tissue culture. Conjunctival scrapings for *C. trachomatis* can be prepared for immunofluorescent or cytologic examination and for appropriate culture. Infections of internal sites pose a more difficult problem. Some, such as acute endophthalmitis, may require removal of infected aqueous humor for microbiologic studies. Infections involving the uveal tract may require indirect methods of diagnosis, such as serologic tests for toxoplasmosis and deep mycoses, blood cultures to demonstrate evidence of disseminated disease (eg, *Candida* sepsis), and efforts to demonstrate infection in other sites (eg, chest radiography and sputum culture to diagnose tuberculosis). Careful ophthalmologic examination using slit lamps and retinoscopy often helps suggest specific etiologic agents based on the morphology of the lesions observed.

Gram stain and cultures of surface scrapings

Most agents can be cultured

## MANAGEMENT PRINCIPLES

Various topical antimicrobial agents have been used effectively in external eye infections of presumed or proved bacterial origin. In addition, topical antiviral treatment is available for herpes simplex infections but has not been proved efficacious for other viral diseases of the eye. Severe infections, whether external or internal, require specialized treatment that nearly always includes ophthalmologic consultation because they may threaten vision. Systemic infection associated with eye disease (eg, fungemia, tuberculosis) must be treated vigorously with appropriate antimicrobial agents.

Topical agents used for superficial bacterial and herpes simplex infections

Ophthalmologic consultation needed with severe or deep infection

# EAR INFECTIONS

Most infections of the ear involve the external otic canal (otitis externa) or the middle ear cavity (otitis media), which contains the ossicles and is enclosed by bony structures and the tympanic membrane. Factors of importance in the pathogenesis of otitis externa include local trauma, furunculosis, foreign bodies, or excessive moisture, which can lead to maceration of the external ear epithelium (swimmer's ear). Occasionally, external otitis occurs as an extension of infection from the middle ear, with purulent drainage through a perforated tympanic membrane.

Otitis externa linked to ear canal trauma and excessive moisture

The eustachian tube, which vents the middle ear to the nasopharynx, appears to play a major role in predisposing patients to otitis media. The tube performs three functions: ventilation, protection, and clearance via mucociliary transport. Viral upper respiratory infections or allergic conditions can cause inflammation and edema in the eustachian tube

Viral infections and allergy predispose to otitis media

Microbes enter middle ear by the eustachian tube

Failure to clear leads to otitis media

or at its orifice. These developments disturb its functions, of which ventilation may be the most important. As ventilation is lost, oxygen is absorbed from the air in the middle ear cavity, producing negative pressure. This pressure in turn allows entry of potentially pathogenic bacteria from the nasopharynx into the middle ear, and failure to clear these normally can result in colonization and infection. Other factors that can lead to compromise of eustachian tube function include anatomic abnormalities, such as tissue hypertrophy or scarring around the orifice, muscular dysfunction associated with cleft palate, and lack of stiffness of the tube wall. The latter is common in infancy and early childhood and improves with age. It may explain in part why otitis media occurs most often in infants 6 to 18 months of age and then decreases in frequency as patency of the eustachian tube becomes established.

## MANIFESTATIONS

*P. aeruginosa* causes swimming pool and malignant otitis externa

Otitis externa is characterized by inflammation of the ear canal, with purulent ear drainage. It can be quite painful, and cellulitis can extend into adjacent soft tissues. A common form is associated with swimming in water that may be contaminated with aerobic, Gram-negative organisms such as *Pseudomonas* species. "Malignant" otitis externa is a considerably more severe form of external ear canal infection that can progress to invasion of cartilage and adjacent bone, sometimes leading to cranial nerve palsy and death. It is seen most frequently in elderly patients with diabetes mellitus and in immunocompromised hosts of any age. *Pseudomonas aeruginosa* is the most common causative pathogen.

Acute otitis media usually bacterial

Extension to deeper structures leads to mastoiditis and sometimes CNS involvement

Otitis media is arbitrarily classified as acute, chronic, or serous (secretory). Acute otitis media, nearly always caused by bacteria, is often a complication of acute viral upper respiratory illness. Fever, irritability, and acute pain are common, and otoscopic examination reveals bulging of the tympanic membrane, poor mobility, and obscuration of normal anatomic landmarks by fluid and inflammatory cells under pressure. In some cases, the tympanic membrane is also acutely inflamed, with blisters (bullae) on its external surface (myringitis). If treated inadequately, the infection can progress to involve adjacent structures such as the mastoid air cells (mastoiditis) or lead to perforation with spontaneous drainage through the tympanic membrane. Potential acute, suppurative sequelae include extension into the central nervous system (CNS) and sepsis.

Chronic otitis media follows unresolved acute infections

Chronic otitis media is usually a result of acute infection that has not resolved adequately, either because of inadequate treatment in the acute phase or because of host factors that perpetuate the inflammatory process (eg, continued eustachian tube dysfunction, caused by allergic or anatomic factors or immunodeficiency). Sequelae include progressive destruction of middle ear structures and a significant risk of permanent hearing loss. Serous otitis media may represent either a form of chronic otitis media or allergy-related inflammation. It tends to be chronic, causing hearing deficits, and is associated with thick, usually nonpurulent secretions in the middle ear.

## COMMON ETIOLOGIC AGENTS

*S. pneumoniae* most common cause

*H. influenzae* strains usually nontypeable

The usual causes of ear infections are listed in Table 61–2. *S. pneumoniae* is the single most common cause of acute otitis media after the first 3 months of life, accounting for 35 to 40% of all cases. *H. influenzae* is also common, particularly in patients less than 5 years of age. The majority of *H. influenzae* isolates from the middle ear are nontypeable; thus the current vaccine against type b strains would not be expected to markedly reduce the incidence of acute otitis media. Viruses and *Mycoplasma* are rare primary causes of acute or chronic otitis media; however, they predispose patients to superinfection by the bacterial agents.

## DIAGNOSTIC APPROACHES

The diagnosis is established on the basis of clinical examination. Tympanometry can be performed in suspected cases of otitis media to detect the presence of fluid in the middle ear and to assess tympanic membrane function. The specific etiology of otitis externa can

TABLE 61–2

**Common Causes of Ear Infection**

| Disease | Cause |
|---|---|
| Otitis externa | *Pseudomonas aeruginosa* is common; occasionally *Proteus* species, *Escherichia coli,* and *Staphylococcus aureus;* bacteria found in otitis media may also be recovered if the process is secondary to middle ear infection with perforation and drainage through the tympanic membrane; fungi, such as *Aspergillus* species, are occasionally implicated |
| Acute otitis media | |
| <3 mo of age | *Streptococcus pneumoniae,* group B streptococci, *Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa,* and Gram-negative enteric bacteria |
| >3 mo of age | *Streptococcus pneumoniae* and *Haemophilus influenzae* are most common; others include *Streptococcus pyogenes, Moraxella catarrhalis,* and *Staphylococcus aureus* |
| Chronic otitis media | Mixed flora in 40% of cases cultured. Common organisms include *Pseudomonas aeruginosa, Haemophilus influenzae, Staphylococcus aureus, Proteus* species, *Klebsiella pneumoniae, Moraxella catarrhalis,* and Gram-positive as well as Gram-negative anaerobic bacteria |
| Serous otitis media | Same as chronic otitis media; however, many more of these effusions are sterile, with relatively few acute inflammatory cells |

be determined by culture of the affected ear canal; however, one must keep in mind that surface contamination and normal skin flora may lead to mixed cultures, which can be confusing. In otitis media, the most precise diagnostic method is careful aspiration with a sterile needle through the tympanic membrane after decontamination of the external canal. Gram stain and culture of such aspirates is highly reliable; however, this procedure is generally reserved for cases in which etiologic possibilities are extremely varied, as in young infants, or when clinical response to the usual antimicrobial therapy has been inadequate. Respiratory tract cultures, such as those from the nasopharynx, cannot be relied on to provide an etiologic diagnosis.

External ear canal cultures often confusing

Middle ear aspirate cultures reliable but reserved for difficult cases

Respiratory tract cultures unhelpful

## MANAGEMENT PRINCIPLES

Except in severe cases, otitis externa can usually be managed by gentle cleansing with topical solutions. The Gram-negative bacteria most commonly involved are often susceptible to an acidic environment, and otic solutions buffered to a low pH (3.0 or less), as with 0.25% acetic acid, are often effective. Various preparations are available, many of which also contain antimicrobics.

Otitis externa treated with topical agents

Acute otitis media requires antimicrobial therapy and careful follow-up to ensure that the disease has resolved. The choice of antimicrobic is usually empirical, designed specifically to cover the most likely bacterial pathogens, because direct aspiration for diagnostic purposes is usually unnecessary. In the usual case, these pathogens would be *S. pneumoniae* and *H. influenzae.* If there is extreme pressure with severe pain, drainage of middle ear exudates by careful incision of the tympanic membrane may be necessary. In patients with chronic or serous otitis media, management can be more complex, and it is often advisable to seek otolaryngologic consultation to determine further diagnostic procedures as well as to plan medical and possible surgical measures.

Antimicrobic therapy for otitis media directed at common agents for age group

Drainage may be required

# SINUS INFECTIONS

The paranasal sinuses (ethmoid, frontal, and maxillary) all communicate with the nasal cavity. In healthy individuals, these sinuses are air-filled cavities lined with ciliated epithelium and are normally sterile. They are poorly developed in early life and, in contrast to otitis media, sinus infections are a rare problem in infancy. The pathogenesis of sinus infection can involve several factors, most of which act by producing obstruction or edema of the sinus opening, impeding normal drainage. Consequently, bacterial infection and inflammation of the mucosal lining tissues develop. Predisposing factors may be (1) local, such as upper respiratory infections producing edema of antral tissues, mucosal polyps, deviation of the nasal septum, enlarged adenoids, or a tumor or foreign body in the nasal cavity; or (2) systemic, such as allergy, cystic fibrosis, or immunodeficiency. Occasionally, maxillary sinusitis can result from extension of a maxillary dental infection.

Factors predisposing to sinusitis involve obstruction of drainage or extension from other sites

## MANIFESTATIONS

Signs and symptoms vary according to which sinuses are affected and whether the illness is acute or chronic. Fever is sometimes present. In addition, nasal or postnasal discharge, daytime cough that may become worse at night, fetid breath, pain over the affected sinus, headache, and tenderness to percussion over the frontal or maxillary sinuses are all features that may appear in different combinations and suggest the diagnosis. Complications of sinusitis can include extension of infection to nearby soft tissues, such as the orbit, and occasionally spread, either directly or via vascular pathways, into the CNS.

Fever and tenderness in local area are common

## COMMON ETIOLOGIC AGENTS

Table 61–3 summarizes the usual etiologies of sinus infections. Respiratory viruses are also occasional direct causes but are most important as predisposing factors to bacterial superinfection of inflamed sinuses and their antral openings. Together, *S. pneumoniae* and *H. influenzae* account for more than 60% of cases of acute sinusitis. Opportunistic, saprophytic fungi, such as *Mucor, Aspergillus,* and *Rhizopus* species, are being increasingly seen in compromised hosts, such as those with severe diabetes mellitus or immunodeficiency. These have a particular tendency to spread progressively to adjacent tissues and to the CNS and are very difficult to treat.

Opportunistic fungi are increasingly found in immunocompromised patients

## DIAGNOSTIC APPROACHES

Radiographic studies of the sinuses confirms the diagnosis. If it becomes necessary to determine the specific infectious agent, fluid should be obtained directly from the affected sinus by needle puncture of the sinus wall or by catheterization of the sinus antrum after careful decontamination of the entry site. Gram smears and cultures are then made. Cultures of drainage from the antral orifices or nasal secretions are unreliable because of contaminating aerobic and anaerobic normal flora.

Gram stain and cultures of direct sinus aspirates most accurate

Cultures of sinus drainage unreliable

TABLE 61–3

**Common Causes of Sinus Infection**

| Disease | Cause |
|---|---|
| Acute sinusitis | *Streptococcus pneumoniae* and *Haemophilus influenzae* are most common; also *Streptococcus pyogenes, Staphylococcus aureus,* and *Moraxella catarrhalis* |
| Chronic sinusitis | Same as for acute sinusitis; also Gram-negative enteric bacteria and anaerobic Gram-negative and Gram-positive bacteria; mixed aerobic and anaerobic infections are relatively common; opportunistic fungi may be found in compromised patients (eg, those with diabetes mellitus) |

## MANAGEMENT PRINCIPLES

In uncomplicated acute sinusitis, prompt antimicrobial therapy is initiated. The choice of antimicrobics is usually empirical, based on the most likely bacterial causes and their usual susceptibility. For example, amoxicillin is effective against most strains of *S. pneumoniae* and *H. influenzae.* Severe, complicated acute infections and chronic sinusitis often require otolaryngologic consultation. In such cases, it is often necessary to obtain cultures directly from the sinuses to select specific antimicrobial therapy, consider the need for surgical procedures to adequately remove the pus and inflammatory tissues, and correct any anatomic obstruction that may exist.

Antimicrobic choice is usually empirical in uncomplicated cases

Direct cultures may be required in severe, chronic cases

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CHAPTER 62

# Dental and Periodontal Infections

Murray R. Robinovitch

Dental caries, chronic periodontitis, and the sequelae of these two diseases constitute the majority of oral and dental infections and the cause of tooth loss. In both, the source of the causative bacteria is the microbial plaque that forms on the teeth. Thus, although dental caries and chronic periodontitis are distinctly different, the prevention and/or halting of the progression of these diseases relies on the elimination of dental plaque from the tooth surfaces. In addition to causing caries and chronic periodontitis, the bacteria of dental plaque play a role in more aggressive forms of periodontitis and necrotizing periodontal diseases.

**Dental plaque** is a soft, adherent dental deposit that forms as a result of bacterial colonization of the tooth surface. It is rather insoluble, as well as adherent, and thus resists removal by water spray or mouth rinsing. Only more vigorous means such as tooth brushing and flossing between the teeth remove it. It consists almost entirely of bacterial cells ($1.7 \times 10^{11}$ cells/g wet weight).

Dental plaque is a deposit from bacterial colonization

**Dental caries** is the progressive destruction of the mineralized tissues of the tooth, primarily caused by the production of organic acids resulting from the glycolytic metabolic activity of plaque bacteria. The basic characteristic of the carious lesion is that it progresses inward from the tooth surface, either the enamel-coated crown or the cementum of the exposed root surface, involving the dentin and finally the pulp of the tooth (Fig 62–1). From there, infection can extend out into the periodontal tissues at the root apex or apices.

Caries produced by plaque bacteria

Plaque-induced periodontal disease encompasses two separate disease entities: gingivitis and chronic periodontitis. These diseases are believed to be related, in that gingivitis, although a reversible condition, is thought to be an early stage leading ultimately to chronic periodontitis in the susceptible subject. The term **gingivitis** is used when the inflammatory condition is limited to the marginal gingiva and bone resorption around the necks of teeth has not yet begun. **Chronic periodontitis** is used to connote the stage of chronic periodontal disease in which there is progressive loss of tooth support due to resorption of the alveolar bone and periodontal ligament. Periodontitis can also lead to periodontal abscess when the chronic inflammatory state around the necks of the teeth becomes acute at a specific location.

Chronic periodontal infection causes destruction of supporting tissues

**Chronic periodontitis,** formerly referred to as adult periodontitis, is responsible for most tooth loss in people greater than 35 to 40 years of age. The term chronic indicates that the disease progresses slowly and results in the progressive destruction of the supporting tissues of the tooth (periodontal ligament and alveolar bone) from the margins of the gingiva toward the apices of the roots of the teeth. Although the accumulative effects

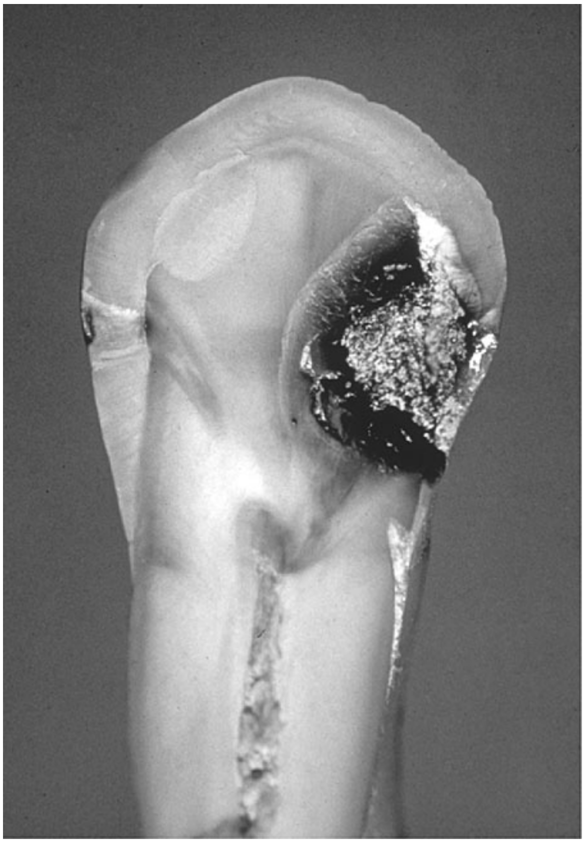

FIGURE 62–1
Hemisected human tooth showing an advanced carious lesion on the right side of the crown and a much smaller lesion on the left side. Note the progression of the lesion through the enamel and dentin, pointing toward the pulp chamber in the center of the tooth.

of the disease make it appear chronic in nature, the disease may occur as a series of acute episodes separated by quiescent periods of indeterminate duration.

Acute periodontitis caused by different organisms

More aggressive forms of periodontitis result in more rapid loss of tooth support. Such aggressive types of disease have been described as localized aggressive periodontitis, which occurs in circumpubertal adolescents (formerly called localized juvenile periodontitis), and generalized aggressive periodontitis, which occurs in young adults (formerly referred to as rapidly progressive or early-onset periodontitis). These diseases are thought to be caused by plaque organisms different from those responsible for chronic marginal periodontitis and/or an altered host resistance to the disease.

## DENTAL PLAQUE

Attachment of bacteria to dental pellicle begins colonization

Adhesion mechanisms are lectin-like

The formation of dental plaque is the result of a very specific colonization of tooth surfaces by oral bacteria. The mineralized tooth surface is always coated with a thin organic film called the dental cuticle or pellicle. This coating results from adsorption and binding of specific salivary macromolecules, mainly proteins and glycoproteins, to the tooth surface. Because this cuticle or pellicle can form in a matter of minutes after the tooth surface is exposed to the oral fluid, bacteria never interact directly with the mineralized tooth surface. Instead, bacterial adherence to the tooth, which begins the colonization of the tooth surface, is mediated by bacterial receptors or adhesins that interact with the pellicle in some fashion, often a specific high affinity mechanism such as a lectin-like interaction. Subsequent to this initial colonization, accumulation of progeny as well as the attachment of other bacterial species occurs via a variety of coaggregation mechanisms.

A number of oral bacteria among the complex indigenous oral flora adhere readily to the cuticle-coated tooth above the gum line or free gingival margin. These are the initiators of plaque formation. Primary among them are Gram-positive cocci, such as the sanguis group of organisms (*Streptococcus sanguis* and related species) and short Gram-positive

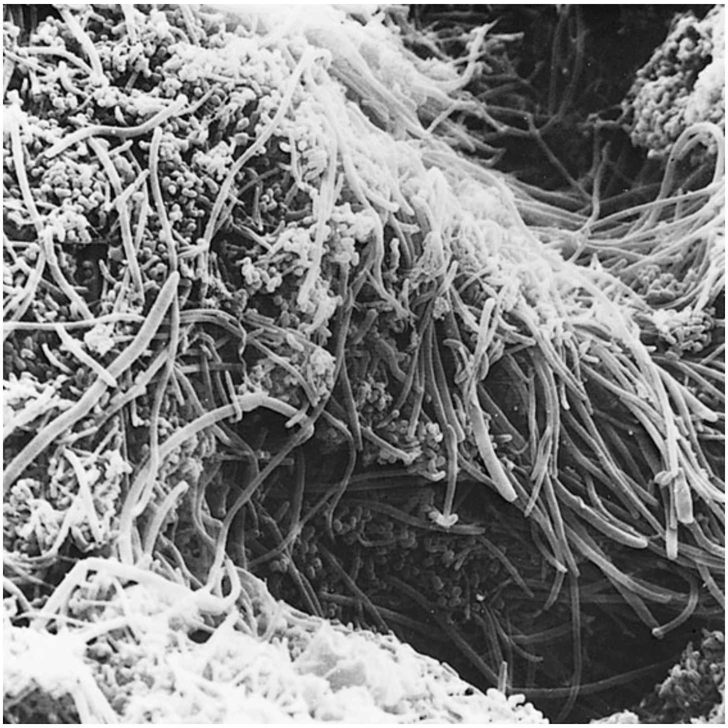

FIGURE 62–2
Scanning electron micrograph of supragingival plaque. (*Courtesy of Dr. W. Fischlsweiger and Dr. Dale Birdsell.*)

rods such as *Actinomyces* species. After 2 to 4 days, fusiform and filamentous organisms appear. Anaerobic vibrios, spirochetes, and a variety of Gram-negative, motile, anaerobic organisms appear at about 6 to 10 days. Thus, as the dental plaque increases in thickness, Gram-negative anaerobic organisms appear and multiply. In all, there are thought to be 300 to 400 bacterial species present in mature dental plaque. The extent and complexity of involved bacteria is shown in Figure 62–2. Dental plaque would coat the tooth surfaces uniformly but for its physical removal during chewing and other oral activities. Characteristically, plaque remains in the non–self-cleansing areas of the teeth such as pits and fissures, along the margins of the gingiva, and between the teeth (Fig 62–3). For this reason, the plaque-related diseases—caries, gingivitis, and periodontitis—occur in their greatest

Plaque comprises many species of bacteria, including anaerobes

Plaque accumulates in non–self-cleansing areas of teeth and along gingival margins

Supragingival and subgingival plaque differ in composition

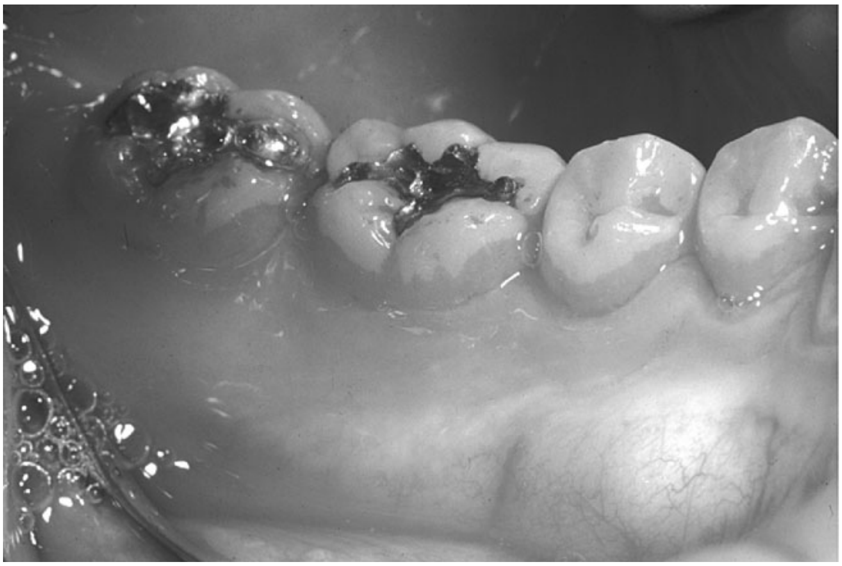

FIGURE 62–3
Dental plaque present along the gingival margins and between the teeth as revealed by erythrocin disclosing dye.

frequency and severity at these locations. In addition to the supragingival plaque, the sulcus around the tooth and periodontal pockets, which are pathologic extensions of the sulcus, are colonized by subgingival bacterial plaque of somewhat different composition. This plaque has a thin adherent layer attached to the tooth surface and a nonadherent bacterial zone between that and the epithelial cells lining the sulcus, containing large numbers of Gram-negative, motile, anaerobic microorganisms. Supragingival plaque lacks such a distinct nonadherent zone. Recent investigations have shown that dental plaque is best described as a biofilm in that its biomass is permeated by fluid-filled channels that transport nutrients, waste products, and other materials, which can act as mediators of many types of interspecies interactions. These interactions are assumed to have a profound influence on the survival and metabolic characteristics of plaque microorganisms.

Removal of plaque prime element of oral hygiene

Chemicals may be used along with brushing and flossing

Because the causative organisms of both dental caries and chronic periodontitis are believed to be in the dental plaque, a prime method for maintaining oral health is regular home care practices for plaque removal. Dental plaque cannot be effectively removed from the teeth solely by chemical or enzymatic means, and the use of antibiotics for prophylactic inhibition of plaque formation cannot be clinically justified, although patients undergoing long-term antibiotic treatment for other medical reasons demonstrate a lower incidence of caries and periodontal disease. Antiseptic substances that bind to tooth surfaces and inhibit plaque formation, such as the bis-biguanides, chlorhexidine and alexidine, have been shown to be effective in reducing plaque, caries, and gingival inflammation. The US Food and Drug Administration has approved a commercial preparation containing 0.12% chlorhexidine for use in controlling dental plaque and associated disease. Toothpaste and mouthrinse additives such as phenolic compounds, essential oils, triclosan, fluorides, herbal extracts, and quaternary ammonium compounds have been shown to have some plaque-reducing ability as well. The use of these substances must be accompanied by proper tooth brushing and flossing to ensure effective disease prevention.

## DENTAL CARIES

Greatest cause of tooth loss in children and young adults

Require microflora and suitable substrates for organic acid production

Dental caries is the single greatest cause of tooth loss in the child and young adult. Its onset can occur very soon after the eruption of the teeth. The first carious lesions usually develop in pits or fissures on the chewing surfaces of the deciduous molars and result from the metabolic activity of the dental plaque that forms in these sites. Later in childhood, the incidence of carious lesions on smooth surfaces increases; these lesions are usually found between the teeth. The factors involved in the formation of a carious lesion are (1) a susceptible host or tooth, (2) the proper microflora on the tooth, and (3) a substrate from which the plaque bacteria can produce the organic acids that result in tooth demineralization.

Saliva protects by mechanical flushing and multiple chemical actions

The newly erupted tooth is most susceptible to the carious process. It gains protection against this disease during the first year or so by a process of posteruptive maturation believed to be attributable to improvement in the quality of surface mineral on the tooth. Saliva provides protection against caries, and patients with dry mouth (xerostomia) suffer from high caries attack rates unless suitable measures are taken. In addition to the mechanical flushing and diluting action of saliva and its buffering capacity, the salivary glands also secrete several antibacterial products. Thus, saliva is known to contain lysozyme, a thiocyanate-dependent sialoperoxidase, and immunoglobulins, principally those of the secretory IgA class. The individual importance of these antibacterial factors is unknown, but they clearly play some role in determining the ecology of the oral microflora.

Fluoride produces more acid-resistant mineral phase of tooth

Proper levels of fluoride, either systemically or topically administered, result in dramatic decreases in the incidence of caries (50 to 60% reduction by water fluoridation, 35 to 40% reduction by topical application). In the case of systemic fluoridation, the protective effect is thought to result from the incorporation of fluoride ions in place of hydroxyl ions of the hydroxyapatite during tooth formation, producing a more perfect and acid-resistant mineral phase of tooth structure. Topical application of fluoride is believed to achieve the same result on the surface of the tooth by initial dissolution of some of the hydroxyapatite, followed by recrystallization of apatite that incorporates fluoride ions into its lattice structure. Another important mode of action, namely, the inhibition of demineralization, and the promotion of remineralization of incipient carious lesions by fluoride ions present in the oral fluid, has more recently been proposed as an important anticaries mechanism of fluoride, perhaps more important than the other proposed mechanisms. In any event, fluoridation represents the most effective means known for rendering the tooth more resistant to the carious process.

The microbial basis of dental caries is well established, and Koch's postulates have been fulfilled, in general, for a number of microorganisms that cause the disease. This confirmation was achieved by using gnotobiotic (sterile) animals whose oral cavities could be colonized with a single organism. At times during the past half-century, a single microorganism was considered responsible for all caries; *Lactobacillus acidophilus* was regarded in this manner in the 1920s, and *Streptococcus mutans* enjoyed this reputation beginning in the 1960s. Currently, it is safe to say that any oral microorganism with a mechanism for colonizing the tooth surface or preexisting plaque and the ability to produce acid (acidogenic) and survive its action (aciduric) can be cariogenic. Organisms isolated from human carious lesions and shown to be cariogenic in gnotobiotic animals include some strains of *S. mutans, Streptococcus salivarius, Streptococcus sanguis, Lactobacillus acidophilus, Lactobacillus casei, Actinomyces viscosus,* and *Actinomyces naeslundii.* However, not all strains of these species are cariogenic in humans.

Members of microflora able to produce acid can be cariogenic

Studies in human subjects indicate that *S. mutans* is a major etiologic agent for smooth surface caries and possibly for pit, fissure, and root surface caries as well. When *S. mutans* strains were collected from different sources and compared, serologic and genetic heterogeneity led to designating some of these as separate species. Currently, those members of the mutans streptococci considered to be important cariogenic organisms in humans are *S. mutans* and *S. sobrinus. Lactobacillus* species may represent secondary invaders of the established caries lesion, but the mutans streptococci are thought to be the main initiators of this disease.

Several species may be cariogenic, but *S. mutans* is most important

Cariogenic organisms must be provided with an appropriate substrate for glycolysis in order to cause tooth demineralization, and dietary monosaccharides and disaccharides such as glucose, fructose, sucrose, lactose, and maltose are readily used by most oral bacteria. These carbohydrates permeate the dental plaque, are absorbed by the bacteria, and are metabolized so rapidly that organic acid products accumulate and cause the pH of the plaque to drop to levels sufficient to demineralize the tooth structure. Production of acid and the decreased pH are maintained until the substrate supply is exhausted. Obviously, foods with a high sugar content that adhere to the teeth and have long oral clearance times are more cariogenic than less retentive foodstuffs such as sugar-containing liquids. Once the substrate is exhausted, the plaque pH returns slowly to its more neutral pH resting level. Frequency of application of substrate is extremely important; the plaque pH may never reach a normal resting level with repeated snacking between meals.

Demineralization is by acid production from dietary carbohydrate breakdown

Degree and duration of acid production facilitated by sticky carbohydrates

Dietary sucrose is also used in the synthesis of extracellular polyglycans such as dextrans and levans by some microorganisms that possess glucose transferase or fructose transferase enzymes on their cell surfaces. Synthesis of polyglycans is considered an additional virulence factor for two reasons:

1. The polyglycan-producing microorganisms are usually aggregated in its presence, which is believed to aid in the colonization and/or accumulation of the organism on the tooth surface. *S. mutans* is a major cariogenic microorganism that acts in this way.
2. Extracellular polyglycan production may increase cariogenicity by serving as an extracellular storage form of substrate. Certain microorganisms synthesize extracellular polyglycan when sucrose is available but then break it down into monosaccharide units to be used for glycolysis when dietary carbohydrate is exhausted. Thus, these microorganisms can prolong acidogenesis beyond the oral clearance time of the substrate.

Extracellular polyglycans synthesized from sucrose important in adherence and carbohydrate storage

Some oral bacteria also use dietary monosaccharides and disaccharides internally to form glycogen, which is stored intracellularly and used for glycolysis after the dietary substrate has been exhausted; thus, the period of acidogenesis is again prolonged and the cariogenicity of the microorganism increased. It is therefore clear that the ability to synthesize extracellular or intracellular storage polysaccharides, to colonize tooth surfaces, and to produce and survive in acid contribute to the microorganism's cariogenicity.

Acidogenesis prolonged from intracellular glycogen stores

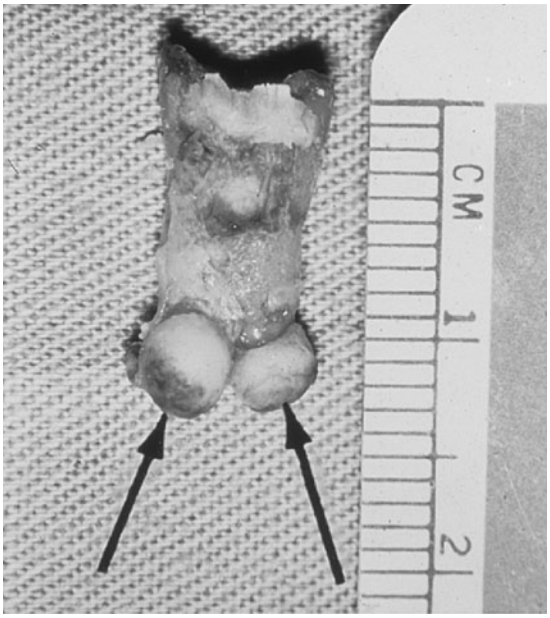


FIGURE 62–4
Periapical involvement of a premolar, resulting from the extension of infection through the root canals and into the periodontium at the root apices. In this case, chronic nonsuppurating lesions have formed at the apical ends of the two root canals (see arrows).

Extension to pulp and periapical locations complicate infections

More severe complications spread to bone or local fascia

The most common complications of dental caries are extension of the infection into the pulp chamber of the tooth (pulpitis), necrosis of the pulp, and extension of the infection through the root canals into the periapical area of the periodontal ligament. Periapical involvement may take the form of an acute inflammation (periapical abscess), a chronic nonsuppurating inflammation (periapical granuloma), or a chronic suppurating lesion that may drain into the mouth or onto the face via a sinus tract (Fig 62–4). A cyst may form within the chronic nonsuppurating lesion as a result of inflammatory stimulation of the epithelial rests normally found in the periodontal ligament. If the infectious agent is sufficiently virulent or host resistance is low, the infection may spread into the alveolar bone (osteomyelitis) or the fascial planes of the head and neck (cellulitis). Alternatively, it may ascend along the venous channels to cause septic thrombophlebitis. Because most carious lesions represent a mixed infection by the time cavities have developed, it is not surprising that most oral infections resulting from the extension of carious lesions are mixed and frequently include anaerobic organisms.

## CHRONIC PERIODONTITIS

Subgingival plaque causes collagen loss

Both gingivitis and chronic periodontitis are now believed to be caused by certain bacteria in the dental plaque that lie in close proximity to the necks of the teeth and marginal gingival tissues. Thus, subgingival plaque found within the gingival crevice or the sulcus around the necks of the teeth is thought to house the etiologic agent(s). The characteristic histopathologic picture of gingivitis is of a marked inflammatory infiltrate of polymorphonuclear leukocytes, lymphocytes, and plasma cells in the connective tissue that lies immediately adjacent to the epithelium lining the gingival crevice and attached to the tooth. Collagen is lost from the inflamed connective tissue. There does not seem to be any direct invasion of the gingival tissues by large numbers of intact bacteria, at least in the early stages of the disease.

It has been proposed that tissue destruction is mediated by bacterial substances that pass through the epithelial barrier and cause either direct or indirect injury. Bacterial products that could cause direct injury to the tissues include toxins, such as endotoxin and leukotoxins, and enzymes, such as hyaluronidase and collagenase. Several mechanisms for indirect injury of the periodontal tissues have been proposed. These hypotheses include initiation of an unresolvable inflammatory response with excessive release of the lysosomal contents from polymorphonuclear leukocytes; activation of complement, which further magnifies the inflammatory response; and development of a host of humoral and cell-mediated immune responses, which can also magnify the inflammatory reaction as well as lead to tissue destruction. A complex pattern of interactions between various released chemokines and cytokines (eg, interleukins 1, 4, 5, 6, and 12; tumor necrosis factor-$\alpha$; interferons-$\alpha$ and $\beta$; transforming growth factor-$\beta$; and prostaglandin $E_2$) and their target cells have been implicated in the modulation of the host response to the periodontal pathogens, some of which may lead to tissue destruction. Many oral bacteria have been found to contain potent polyclonal $\beta$-lymphocyte activators, leading some investigators to propose that periodontal pathogens release these substances into lesions. Polyclonal $\beta$-cell activation could promote an exaggeration of the inflammatory response and further tissue injury through enhanced antibody and cytokine production. Regardless of the mechanisms of tissue destruction, the true source of the disease, namely the causative bacteria, remains outside the gingival tissues in supra- and subgingival plaque and is therefore often resistant to the body's defense mechanisms. There is evidence that some bacteria do invade the gingival tissues, especially in the more aggressive forms of periodontitis, and this invasion may constitute a pathogenic mechanism. Nevertheless, the origin of these bacteria is the dental plaque, and so the disease continues to progress unless the dental plaque is removed and the involved tooth is kept plaque-free. If these measures are taken, chronic gingivitis can resolve completely and the tissues return to normal.

Tissue destruction mediated by bacterial products

Immunologic mediators play a role in tissue damage

Bacterial source of the disease is outside the affected tissues

As the disease progresses, a point may be reached at which the alveolar bone around the necks of the teeth is resorbed; the condition is then no longer termed gingivitis, but periodontitis. With resorption of the bone, the attachment of the periodontal ligament is lost and the gingival sulcus deepens into a periodontal pocket. Periodontitis is not considered to be a reversible disease in that the lost alveolar bone and periodontal ligament do not regenerate with cessation of the inflammation, even though further progression may be halted. If unchecked, bone resorption progresses to loosening of the tooth, which may ultimately be exfoliated. Figure 62–5 shows a case of advanced chronic periodontitis in which the gingival tissues are inflamed, gross deposits of plaque and calculus around the necks of the teeth are apparent, and the teeth have spread apart and extruded due to the major loss in their periodontal attachment. Occasionally, the neck of a periodontal pocket becomes constricted, the bacteria proliferate causing an acute inflammatory response in the occluded pocket, and a periodontal abscess results. This acute exacerbation requires drainage in the same way as abscesses elsewhere for the patient to obtain symptomatic relief.

With continued progress, periodontitis and bone resorption develop

Periodontal abscess may result

Gingivitis develops within 2 weeks in individuals who fail to practice effective tooth cleansing. It is not known whether particular species of plaque bacteria are responsible for gingival inflammation, but among those suspected of pathogenicity in the case of

Multiple organisms involved in chronic periodontitis

FIGURE 62–5
A patient exhibiting advanced chronic periodontitis as evidenced by marked recession, inflammation of the gingival tissues, and tooth mobility and separation. Note the presence of copious amounts of dental plaque and calculus (mineralized plaque) around the necks of the teeth.

chronic periodontitis are anaerobic Gram-negative rods (*Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus, Campylobacter rectus, Fusobacterium nucleatum*), *Peptostreptococcus micros, Eikenella corrodens,* and *Treponema denticola.* Many of these organisms produce periodontal disease in monoinfected animals. It has been suggested recently that the disease may be caused by the combined effects of two or more of these pathogens at a site, rather than there being only one species of microorganism responsible for the destructive lesion.

Acute juvenile periodontitis associated with *Actinobacillus*

There is some evidence that the causative agents in aggressive forms of periodontitis may differ from those associated with chronic marginal disease. In the condition known as localized aggressive periodontitis, a small capnophilic (carbon dioxide–requiring) Gram-negative rod (*Actinobacillus actinomycetemcomitans*) has been indicted based on studies of the flora of disease sites. A virulence factor found in those strains of *A. actinomycetemcomitans* that are associated with this disease is the production of a leukotoxin by the bacteria. In addition, it has been found that a significant proportion of patients with this condition demonstrate high serum antibody titers to *A. actinomycetemcomitans.* Also of interest is the fact that many of these patients have neutrophil chemotactic or phagocytic defects.

## NECROTIZING PERIODONTAL DISEASES

Acute onset with painful ulcerative lesions

Fusospirochetal etiology together with other anaerobes

Necrotizing ulcerative gingivitis (previously called acute necrotizing ulcerative gingivitis, Vincent's infection, or trench mouth) and necrotizing ulcerative periodontitis represent a spectrum of acute inflammatory disease starting with destruction limited to the soft tissues (gingivitis) and extending to destruction of the alveolar bone and periodontal ligament (periodontitis). This disease spectrum is distinctly different from gingivitis–chronic periodontitis. It has an acute onset, frequently associated with periods of stress and poor oral hygiene. There is rapid ulceration of the interdental areas of the gingiva, resulting in destruction of the interdental papillae. The inflammatory condition initially confined to the gingival tissues can quickly extend into pathologic bone resorption. Unlike gingivitis and chronic periodontitis, acute necrotizing periodontal disease is painful. As the oral epithelium is destroyed, the causative bacteria come into direct contact with the underlying tissues and may invade them. Spirochetes and fusiform bacteria have been implicated; thus, the term fusospirochetal disease has been used to describe this infection, which can also be manifested as ulceration in other areas of the pharynx or oral cavity. *Prevotella intermedia* has also been found in high numbers in the lesions. Morphologic studies have shown that the spirochetes actually appear to invade the tissues. The disease may be treated with systemic antibiotics and topical antimicrobials for immediate relief of symptoms, but resolution is dependent on thorough professional cleaning of the teeth and institution of good home care. Further discussion of fusospirochetal disease is provided in Chapter 27.

## DENTAL PLAQUE AND ORAL FLORA IN THE COMPROMISED PATIENT

Endocarditis from oral flora unless protected by prophylaxis

As it can be the source of transient bacteremia, dental plaque must be viewed as a hazard in the compromised patient. The best example is the patient with heart valve damage as a result of a congenital anomaly, rheumatic fever, or a heart prosthesis. If transient bacteremia develops, the blood-borne bacteria may form vegetative growths in the heart and cause bacterial endocarditis (see Chapter 68). Such patients should always be premedicated with prophylactic antibiotics before any dental procedure with the potential for causing a bacteremia is performed, including routine dental prophylaxis.

Severe opportunistic infections may develop in the immunocompromised patient

It has also been established that dental plaque organisms and other oral bacteria may give rise to serious systemic infections in patients whose host defense mechanisms are compromised. Patients who have undergone extensive radiation treatment of the jaw area, for example, are prone to develop osteomyelitis. Furthermore, one of the most frequent sources of fatal infections in leukemic patients is the oral cavity. Therefore, for these patients scrupulous home care and professional dental treatment are indicated prior to undergoing immunosuppressive therapies.

## ADDITIONAL READING

The following references are authoritative reviews of caries and periodontal disease and new advances in understanding these conditions:

Newbrun E. *Cariology,* 3rd ed. Chicago: Quintessence Publishing; 1989.

Newman MG, Takei HH, Carranza FA (eds). *Carranza's Clinical Periodontology.* 9th ed. Philadelphia: WB Saunders; 2002.

Socransky SS, Haffajee AD (eds). Microbiology and immunology of periodontal diseases. In *Periodontology 2000,* vol 5. Copenhagen, Denmark: Munksgaard; 1994.

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CHAPTER 63

# Upper Respiratory Tract Infections and Stomatitis

C. George Ray

Upper respiratory infections usually involve the nasal cavity and pharynx, and most (more than 80%) are caused by viruses. Like middle and lower respiratory illnesses, the diseases of the upper respiratory tract are named according to the anatomic sites primarily involved. **Rhinitis** (or coryza) implies inflammation of the nasal mucosa, **pharyngitis** denotes pharyngeal infection, and **tonsillitis** indicates an inflammatory involvement of the tonsils. Because of the close proximity of these structures to one another, infections may simultaneously involve two or more sites (eg, rhinopharyngitis or tonsillopharyngitis). All such infections are grouped under the general term upper respiratory infections. **Stomatitis** is a term used to describe infections primarily localized to the mucous membranes of the oral cavity. These infections can sometimes also involve the tongue (**glossitis**) or the gingival and periodontal tissues (**gingivostomatitis** or acute necrotizing ulcerative gingivitis; see Chapter 62).

Most upper respiratory infections are caused by viruses

Other infections considered are peritonsillar abscess (quinsy), retrotonsillar abscess, and retropharyngeal abscess. These infections are the result of direct invasion from mucosal sites and localization in deeper tissues to produce inflammation and abscess formation.

## CLINICAL FEATURES

**Rhinitis** is the most common manifestation of the common cold. It is characterized by variable fever, inflammatory edema of the nasal mucosa, and an increase in mucous secretions. The net result is varying degrees of nasal obstruction; the nasal discharge may be clear and watery at the onset of illness, becoming thick and sometimes purulent as the infection progresses over 5 to 10 days.

The common cold is characterized by rhinitis

**Pharyngitis** and **tonsillitis** are associated with pharyngeal pain (sore throat) and the clinical appearance of erythema and swelling of the affected tissues. There may be exudates, consisting of inflammatory cells overlying the mucous membrane, and petechial hemorrhages; the latter may be seen in viral infections but tend to be more prominent in bacterial infections. Viral infections, particularly herpes simplex, may also lead to the formation of vesicles in the mucosa, which quickly rupture to leave ulcers. Pharyngeal candidiasis can also erode the mucosa under the plaques of "thrush." On rare occasions, the local inflammation may be sufficiently severe to produce **pseudomembranes,** which

Inflammatory exudates and hemorrhages more common in bacterial infections

Vesicles and ulcerated lesions more common in viral disease

Pharyngeal pseudomembranes in diphtheria

consist of necrotic tissue, inflammatory cells, and bacteria. This finding is particularly common in pharyngeal diphtheria, but may be mimicked by fusospirochetal infection (Vincent's angina) and sometimes by infectious mononucleosis. In acute tonsillitis or pharyngitis of any etiology, regional spread of the infecting agents with inflammation and tender swelling of the anterior cervical lymph nodes is also common.

Herpes and *Candida* most common causes of stomatitis

**Stomatitis** is inflammation of the oral cavity. Multiple ulcerative lesions of the oral mucosa, seen most frequently with severe primary herpes simplex infections, may extend to the tongue, lips, and face. In extreme cases, the pain may be so severe that the patient requires relief with topical anesthetics during the usual 9- to 12-day period of acute symptoms. *Candida* species can also invade oral surfaces to produce plaques identical to those of pharyngeal thrush. This infection is particularly common in young infants and immunocompromised individuals of any age.

Aphthous stomatitis (canker sores) have unknown cause

**Aphthous stomatitis** is a recurrent disease of the oral mucosa characterized by single or multiple painful ulcers with irregular margins, usually 2 to 10 mm in diameter. Healing usually occurs in a few days. The term commonly used to describe this condition is **canker sore.** The cause is unknown. It can easily be confused with recurrent herpes simplex lesions and, like herpes, tends to recur in relation to stress, menses, local trauma, and other nonspecific stimuli.

Noma is an extensive stomatitis of debilitated persons

Mild stomatitis occurs with many viral infections

A severe, gangrenous stomatitis that progresses beyond the mucous membranes to involve soft tissues, skin, and sometimes bone can complicate a variety of acute illnesses in patients who are severely debilitated and whose oral hygiene is poor. This infection, called **noma** or **cancrum oris,** is rarely seen in the United States. Typical cases occur among children with severe protein–calorie malnutrition or other immune compromise. Measles sometimes precipitates noma. Etiologic agents thought to be involved include *Fusobacterium* and *Bacteroides* species, as well as *Pseudomonas aeruginosa.* Milder forms of stomatitis are seen in a variety of other common viral infections. Examples include Koplik's spots in measles, buccal or palatal ulcers in chickenpox, and similar phenomena in some enteroviral infections such as hand, foot, and mouth disease.

Tonsillar asymmetry a sign of peritonsillar abscess

Peritonsillar or retrotonsillar abscesses are usually a complication of tonsillitis. They are manifested by local pain, and examination of the pharynx reveals tonsillar asymmetry with one tonsil usually displaced medially by the abscess. This infection is most common in children more than 5 years of age and in young adults. If not properly treated, the abscess may spread to adjacent structures. It can involve the jugular venous system, erode into branches of the carotid artery to cause acute hemorrhage, or rupture into the pharynx to produce severe aspiration pneumonia.

Retropharyngal abscess causes anterior bulging of pharyngeal wall

Retropharyngeal or lateral pharyngeal abscesses occur most frequently in infants and children less than 5 years of age. They can result from pharyngitis or from accidental perforation of the pharyngeal wall by a foreign body. The infection is characterized by pain, inability or unwillingness to swallow, and, if the pharyngeal wall is displaced anteriorly near the palate, a change in phonation (nasal speech). The neck may be held in an extended position to relieve pain and maintain an open upper airway. Examination of the pharynx usually reveals anterior bulging of the pharyngeal wall; if this finding is not apparent, lateral x-rays of the neck may demonstrate a widening of the space between the cervical spine and the posterior pharyngeal wall. The complications of such abscesses are basically the same as those described for peritonsillar abscesses; in addition, the suppurative process can extend posteriorly to the cervical spine to produce osteomyelitis or inferiorly to cause acute mediastinitis.

Oral and pharyngeal lesions accentuated in immunocompromised hosts

May be portal of entry for systemic infection

In the immunocompromised patient, all of the various forms of stomatitis and pharyngitis described previously can be accentuated. Leukemia, agranulocytosis, chronic ulcerative colitis, congenital or acquired immunodeficiency (eg, AIDS), and treatment with cytotoxic or immunosuppressive drugs are commonly associated with such lesions. The marked damage to mucosal tissues that sometimes occurs can provide a portal of entry into deeper structures and then to the systemic circulation, creating a risk of bacterial or fungal sepsis. Conversely, oral lesions may also result from dissemination of infection from other remote sites. Examples include disseminated histoplasmosis and sepsis caused by *Pseudomonas* species.

TABLE 63–1

**Major Infectious Causes of Upper Respiratory Disease**

| Disease | Viruses | Bacteria and Fungi |
|---|---|---|
| Rhinitis | Rhinoviruses, adenoviruses, coronaviruses, parainfluenza viruses, influenza viruses, respiratory syncytial virus, some coxsackie A viruses | Rare |
| Pharyngitis or tonsillitis | Adenoviruses, parainfluenza viruses, influenza viruses, rhinoviruses, coxsackie A or B virus, herpes simplex virus, Epstein–Barr virus | Group A streptococcus (*S. pyogenes*) *Corynebacterium diphtheriae, Neisseria gonorrhoeae* |
| Stomatitis | Herpes simplex virus, some coxsackie A viruses | *Candida* species, *Fusobacterium* species, spirochetes |
| Peritonsillar or retropharyngeal abscess | None | Group A streptococcus (most common), oral anaerobes such as *Fusobacterium* species, *Staphylococcus aureus, Haemophilus influenzae* (usually in infants) |

## COMMON ETIOLOGIC AGENTS

Table 63–1 lists the more common causes of upper respiratory infections and stomatitis. Viral infections predominate. The most frequent bacterial cause to be considered is *S. pyogenes*. *Corynebacterium diphtheriae,* although rare in the United States, is a major pathogen that continues to cause infection in many other countries and must not be overlooked, particularly if clinical and epidemiologic findings suggest this possibility. *Neisseria gonorrhoeae,* isolated from adults with symptomatic pharyngitis in whom no other etiologic agent can be demonstrated, is now considered a pharyngeal pathogen that is usually transmitted by oral–genital contact. Occasionally, other bacteria have been implicated as causes of acute pharyngitis (eg, *Corynebacterium ulcerans, Arcanobacterium haemolyticum, Francisella tularensis,* and streptococci of groups B, C, and G). These are listed here for the sake of completeness but are not routinely sought except in unusual circumstances.

Viral infections predominate

*S. pyogenes* and *C. diphtheriae* are bacterial pathogens

Gonococcal pharyngitis occurs with oral–genital contact

In patients with purulent rhinitis, sinusitis should also be considered in the differential diagnosis (see Chapter 61). Unilateral and foul-smelling purulent discharge suggests the presence of a foreign body in the nose.

## GENERAL DIAGNOSTIC APPROACHES

Although viruses cause the vast majority of upper respiratory infections, they are generally not amenable to specific therapy, and laboratory tests for viral infections are usually reserved for investigating outbreaks or in cases in which the illness seems unusually severe or atypical.

The primary diagnostic approach in pharyngitis and tonsillitis is to determine whether there is a bacterial cause requiring specific treatment. The only reliable method is to collect a throat swab for culture, taking care to thoroughly swab the tonsillar fauces as well as the posterior pharynx, and to include any purulent material from inflamed areas. Cultures are usually made only to detect the presence or absence of group A streptococci. Direct antigen tests for rapidly detecting *S. pyogenes* in throat swabs have gained popularity in recent years. These are usually enzyme immunoassay or latex agglutination–based methods. The most common limitation of such tests is lack of sensitivity; that is, false-negative results can occur.

Approach is to determine if there is a bacterial etiology by culture

Direct detection methods have false-negative results

For the laboratory diagnosis of diphtheria or pharyngeal gonorrhea, the clinical suspicion should be indicated to the laboratory so that specific cultures for *C. diphtheriae* or

Evidence for pathogenic role of opportunists assessed by multiple means

Pathogens may be present in normal flora but not cause pharyngitis

*N. gonorrhoeae* may be made. *Candida* species, fusospirochetal bacteria, *Pseudomonas* species, and other Gram-negative organisms are often found in pharyngeal or oral specimens from healthy individuals as well as in certain infections. Their probable pathogenic significance in association with disease in these sites, largely based on the appearance of the lesions and the presence of the organisms in large numbers, can be supported by histologic demonstration of tissue invasion by the organisms. It is important to remember that other bacterial pathogens such as *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae,* and even *Neisseria meningitidis* may be present in the pharynx. These organisms are not primary etiologic agents in rhinitis, pharyngitis, and tonsillitis, and their presence in the throat does not implicate them as causes of the illnesses; they should instead be regarded as colonizers.

The laboratory diagnosis of causes of peritonsillar and retropharyngeal abscesses is based on Gram staining and culture of purulent material obtained directly from the lesion, including anaerobic cultures.

## GENERAL PRINCIPLES OF MANAGEMENT

Penicillins or cephalosporins necessary to treat *S. pyogenes* infections

Macrolides used in penicillin-allergic patients

Viral infections of the upper respiratory tract can only be treated symptomatically. If *S. pyogenes* is the cause, penicillin therapy is required; if the patient is allergic to penicillin, an alternative is chosen (eg, erythromycin or a cephalosporin). Such treatment prevents suppurative or toxigenic complications (eg, pharyngeal abscess, cervical adenitis, and scarlet fever) and the development of acute rheumatic fever. The latter, a serious complication, may occur in 1 to 3% of patients in certain population groups if they are not adequately treated. In addition, treatment of acute streptococcal infections can aid in reducing spread of the organisms to other persons.

Peritonsillar and retropharyngeal abscesses often require surgical drainage

*C. diphtheriae* infections involve more complex management, which includes antitoxin as well as antimicrobic treatment (see Chapter 18). Infections caused by *N. gonorrhoeae* are treated with appropriate antimicrobics (see Chapter 20). The management of stomatitis includes maintenance of adequate oral hygiene. If invasive *Candida* infection is present, topical and/or systemic antifungal therapy is sometimes necessary. Vincent's angina and other fusospirochetal infections are usually treated with systemic penicillin therapy as well as with appropriate dental and periodontal care. There is no specific, widely accepted treatment for aphthous stomatitis. Peritonsillar and retropharyngeal abscesses are treated aggressively with antimicrobics and often require surgical drainage, taking care to prevent accidental aspiration of the abscess contents into the lower respiratory tract.

CHAPTER 64

# Middle and Lower Respiratory Tract Infections

C. GEORGE RAY AND KENNETH J. RYAN

## MIDDLE RESPIRATORY TRACT INFECTION

For the purpose of this discussion, the middle respiratory tract is considered to comprise the epiglottis, surrounding aryepiglottic tissues, larynx, trachea, and bronchi. Inflammatory disease involving these sites may be localized (eg, **laryngitis**) or more widespread (eg, laryngotracheobronchitis). The majority of severe infections occur in infancy and childhood. Disease expression varies somewhat with age, partly because the diameters of the airways enlarge with maturation and because immunity to common infectious agents increases with age. For example, an adult with a viral infection of the larynx (laryngitis) who was exposed to the same virus in childhood has a relatively better immune response; in addition, the larger diameter of the larynx in the adult permits greater air flow in the presence of inflammation. An infant or child with the same infection in the same site can develop a much more severe illness, known as **croup,** which can lead to significant obstruction of air flow.

Most severe middle tract infections occur in infancy and childhood

### CLINICAL FEATURES

**Epiglottitis** is often characterized by the abrupt onset of throat and neck pain, fever, and inspiratory stridor (difficulty in moving adequate amounts of air through the larynx). Because of the inflammation and edema in the epiglottis and other soft tissues above the vocal cords (supraglottic area), phonation becomes difficult (muffled phonation or aphonia), and the associated pain leads to difficulty in swallowing. If this disease is not treated promptly, death may result from acute airway obstruction.

Epiglottitis carries risk of acute airway obstruction

Laryngitis or its more severe form, croup, may have an abrupt onset (spasmodic croup) or develop more slowly over hours or a few days as a result of spread of infection from the upper respiratory tract. The illness is characterized by variable fever; inspiratory stridor; hoarse phonation; and a harsh, barking cough. In contrast to epiglottitis, the inflammation is localized to the subglottic laryngeal structures, including the vocal cords. It sometimes extends to the trachea (laryngotracheitis) and bronchi (laryngotracheobronchitis), where it is associated with a deeper, more severe cough that may provoke chest pain

Laryngitis and croup involve subglottic laryngeal structures and variable degrees of sputum production. When vocal cord inflammation is severe, transient aphonia may result.

Bronchitis involves larger airways

**Bronchitis** or **tracheobronchitis** may be a primary manifestation of infection or a result of spread from upper respiratory tissues. It is characterized by cough, variable fever, and sputum production, which is often clear at the onset but may become purulent as the illness persists. Auscultation of the chest with the stethoscope often reveals coarse bubbling rhonchi, which are a result of inflammation and increased fluid production in the larger airways.

Chronic bronchitis associated with smoking, air pollution, and other diseases

Nontypeable *H. influenzae* and *S. pneumoniae* found in exacerbations of chronic bronchitis

**Chronic bronchitis** is a result of long-standing damage to the bronchial epithelium. A common cause is cigarette smoking, but a variety of environmental pollutants, chronic infections (eg, tuberculosis), and defects that hinder normal clearance of tracheobronchial secretions and bacteria (eg, cystic fibrosis) can be responsible. Because of the lack of functional integrity of their large airways, such patients are susceptible to chronic infection with members of the oropharyngeal flora and to recurrent, acute flare-ups of symptoms when they become colonized and infected by viruses and bacteria, particularly *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae.* A vicious cycle of recurrent infection may evolve, leading to further damage and increasing susceptibility to pneumonia.

## COMMON ETIOLOGIC AGENTS

Most subglottic middle airway infections are viral

With the exception of epiglottitis, acute diseases of the middle airway are usually caused by viral agents (Table 64–1). When acute airway obstruction is present, noninfectious possibilities, such as aspirated foreign bodies and acute laryngospasm or bronchospasm caused by anaphylaxis, must also be considered.

## GENERAL DIAGNOSTIC APPROACHES

When a viral etiology is sought, the usual method of obtaining a specific diagnosis is by inoculation of cell cultures with material from the nasopharynx and throat. Acute and

TABLE 64–1

**Major Causes of Acute Middle Respiratory Tract Disease**

| Syndrome | Viruses | Bacteria | Percentage Caused by Viruses |
|---|---|---|---|
| Epiglottitis | Rare | *Haemophilus influenzae, Streptococcus pneumoniae, Corynebacterium diphtheriae, Neisseria meningitidis* | 10 |
| Laryngitis and croup | Parainfluenza viruses, influenza viruses, adenoviruses; occasionally respiratory syncytial virus, rhinoviruses, coronaviruses, echoviruses | Rare | 90 |
| Laryngotracheitis and laryngotracheobronchitis | Same as for laryngitis and croup | *H. influenzae, Staphylococcus aureus* | 90 |
| Bronchitis | Parainfluenza viruses, influenza viruses, respiratory syncytial virus, adenoviruses, measles | *Bordetella pertussis, H. influenzae, Mycoplasma pneumoniae, Chlamydia pneumoniae* | 80 |

convalescent sera can also be collected to determine antibody responses to the common respiratory viruses and *Mycoplasma pneumoniae.* In bacterial infections, the approaches noted below are valuable.

## Epiglottitis

*H. influenzae* type b, once the most common cause of epiglottitis, produces an associated bacteremia in 85% of cases or more. Attempts to obtain cultures from the epiglottis or throat may provoke acute reflex airway obstruction in patients who have not undergone intubation to ensure proper ventilation; furthermore, the yield is lower than that of blood culture. In addition, other bacterial agents that cause epiglottitis can often be isolated from the blood. The exception is *Corynebacterium diphtheriae* infection, in which cultures of the nasopharynx or pharynx are required.

Incidence of bacteremia is high in epiglottitis

## Laryngotracheitis and Laryngotracheobronchitis

Although most cases of laryngotracheitis and laryngotracheobronchitis have a viral etiology, a severe purulent process is seen occasionally. The latter is often referred to as acute **bacterial tracheitis,** and it can be rapidly fatal if not managed aggressively. Gram staining and culture of sputum, or better yet, of purulent secretions obtained by direct laryngoscopy, help establish the causative agent. Blood cultures are again useful in such cases when a bacterial etiology is suspected.

Bacterial tracheitis is best diagnosed by direct laryngoscopy specimens

## Acute Bronchitis

A major bacteriologic consideration in acute bronchitis, especially in infants and preschool children, is *Bordetella pertussis.* Deep nasopharyngeal cultures plated on the appropriate media constitute the best specimens. Gram staining and examination of nasopharyngeal smears by direct fluorescent antibody methods are also useful adjuncts to establishing the diagnosis. When purulent sputum is produced, Gram staining and culture may be useful in suggesting other bacterial causes (see Table 64–1). Exceptions include *M. pneumoniae* and *Chlamydia pneumoniae* infections, which are usually diagnosed by serologic testing of acute and convalescent sera.

Nasopharyngeal specimens are appropriate for diagnosis of pertussis

Serodiagnosis commonly used for *M. pneumoniae* and *C. pneumoniae* infections

## GENERAL PRINCIPLES OF MANAGEMENT

The primary initial concern is ensuring an adequate airway. It is particularly crucial in epiglottitis but can also become a major issue in laryngitis or laryngotracheobronchitis. Thus, some patients require placement of a rigid tube that provides communication between the tracheobronchial tree and the outside air (a nasotracheal tube or a surgically placed tracheostomy). Other adjunctive measures, such as highly humidified air and oxygen, may also provide relief in acute diseases involving the structures in and around the larynx. In proved or suspected bacterial infections, specific antimicrobic therapy is required; other treatment, such as antitoxin administration in diphtheria, may also be necessary.

Maintenance of airway patency required

Antimicrobic therapy for bacterial infections

# LOWER RESPIRATORY TRACT INFECTION

Lower respiratory tract infection develops with invasion and disease of the lung, including the alveolar spaces and their supporting structure, the interstitium, and the terminal bronchioles. Bronchiolitis, an inflammatory process primarily affecting the small terminal airways in infants, is discussed extensively in Chapter 33. Infection may occur by extension of a middle respiratory tract infection, aspiration of pathogens past the upper airway defenses, or less commonly by hematogenous spread from a distant site such as an abscess or an infected heart valve. When infection develops through the respiratory tract, some compromise of the upper airway mechanisms for filtering or clearing inhaled

Infection can be by inhalation, aspiration, extension from middle tract, or blood-borne infectious agents usually occurs. The most common are those that impair the epiglottic and cough reflexes, such as drugs, anesthesia, stroke, and alcohol abuse. Toxic inhalations and cigarette smoking may also interfere with the normal mucociliary action of the tracheobronchial tree. In healthy persons, the most common antecedent to lower respiratory infection is infection of the middle respiratory structures (usually viral), allowing an otherwise innocuous aspiration of oropharyngeal flora to reach the lower tract and progress to disease rather than undergo rapid clearance. Some small infectious particles can accomplish airborne passage through the middle airway and bypass mucociliary defenses; if they can survive or multiply in alveolar macrophages, they may produce a primary infection. Examples include arthroconidia of *Coccidioides immitis* and cells of *Mycobacterium tuberculosis* and *Bacillus anthracis.*

Infection through air passages is associated with compromised local clearance defenses

## CLINICAL FEATURES

### Acute Pneumonia

Acute pneumonia is an infection of the lung parenchyma that develops over hours to days and, if untreated, runs a natural course lasting days to weeks. The onset may be gradual, with malaise and slowly increasing fever, or sudden, as with the bed-shaking chill associated with the onset of pneumococcal pneumonia. The only early symptom referable to the lung may be cough, which is caused by bronchial irritation. In adults the cough becomes productive of **sputum,** which is purulent material generated in the alveoli and small air passages. In some cases the sputum may be blood streaked, rusty in color, or foul smelling. Labored or difficult breathing (dyspnea), rapid respiratory rate, and sometimes cyanosis are signs of increasing loss of alveolar air-exchange surface through spread of exudate. Chest pain from inflammatory involvement of the pleura is common. Physical signs on auscultation reflect the filling and eventual consolidation of alveoli by fluid and inflammatory cells.

Sputum is purulent material generated in the bronchi and alveoli

Fever, respiratory distress, and sputum production are signs of acute pneumonia

The radiologic pattern of inflammatory changes in the lung is very useful in the diagnosis of pneumonia and for clinical differentiation into likely etiologic categories. The most common pattern is patchy infiltrates related to multiple foci centering on small bronchi (bronchopneumonia), which may progress to a more uniform consolidation of one or more lobes (lobar pneumonia). A more delicate, diffuse, or "interstitial" pattern, which is also common, is particularly associated with viral pneumonia.

Radiologic changes confirm and refine diagnosis

### Chronic Pneumonia

Chronic pneumonia has a slow insidious onset that develops over weeks to months and may last for weeks or even years. The initial symptoms are the same as those of acute pneumonia (fever, chills, and malaise), but they develop more slowly. Cough can develop early or late in the illness. As the disease progresses, appetite and weight loss, insomnia, and night sweats are common. Cough and sputum production may be the first indication of a vague constitutional illness referable to the lung. Bloody sputum (hemoptysis), dyspnea, and chest pain appear as the disease progresses. The physical findings and radiologic features can be similar to those of acute pneumonia, except that the diffuse interstitial infiltrates of viral pneumonia are uncommon. There may be parenchymal destruction and the formation of abscesses or cavities communicating with the bronchial tree. The clinical features of chronic pneumonia may be due to a number of infectious agents or noninfectious causes such as neoplasms, vasculitis, allergic conditions, infarction, radiation or toxic injury, and diseases of unknown etiology (eg, sarcoidosis).

Chronic pneumonia develops over weeks to months

Abscesses and cavities may develop

Chronic pneumonia may have noninfectious causes

**Pleural effusion** is the transudation of fluid into the pleural space in response to an inflammatory process in adjacent lung parenchyma. It may result from a wide variety of causes, both infectious and noninfectious. **Empyema** is a purulent infection of the pleural space that develops when the infectious agent gains access by contiguous spread from an infected lung through a bronchopleural fistula or, less often, by extension of an abdominal infection through the diaphragm. Symptoms are usually insidious and related to the primary infection until enough exudate is formed to produce symptoms referable to the chest wall or to compromise the function of the lung. The physical and radiologic findings are

Pleural effusions may be infectious or noninfectious

Empyema is a purulent infection of pleural space usually by extension of bacterial infection characteristic, with dullness to percussion and localized opacities on x-ray. In contrast to noninfectious effusions, empyema is frequently loculated.

### Lung Abscess

Lung abscess is usually a complication of acute or chronic pneumonia caused by organisms that can cause localized destruction of lung parenchyma. It may occur as part of a chronic process or as an extension of an acute, destructive pneumonia, often after aspiration of oral or gastric contents. The symptoms of lung abscess, which are usually not specific, resemble those of chronic pneumonia or an acute pneumonia that has failed to resolve. Persistent fever, cough, and the production of foul-smelling sputum are typical. Lung abscess can be diagnosed and localized with certainty only radiologically; it appears as a localized area of inflammation with single or multiple excavations or as a cavity with an air–fluid level. Multiple abscesses may develop as a result of blood-borne infection.

Lung abscess frequently follows aspiration pneumonia

Blood-borne infection may cause multiple abscesses

## COMMON ETIOLOGIC AGENTS

The infectious agents that most frequently cause lower respiratory infection are listed in Table 64–2. The etiology of acute pneumonia is strongly dependent on age. More than 80% of pneumonias in infants and children are caused by viruses, whereas less than 10 to 20% of pneumonias in adults are viral. The reasons are probably the same as those indicated previously for middle respiratory tract infections. Influenza and other viruses,

Most pneumonias are viral in infants and children

Viral infections predispose to acute bacterial pneumonia

TABLE 64–2

**Major Causes of Lower Respiratory Tract Infection**

| Syndrome | Viruses | Common Bacteria | Fungi | Other Agents |
|---|---|---|---|---|
| Acute pneumonia | Influenza,[a] parainfluenza, adenovirus, respiratory syncytial virus (infants)[a] | *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae,* Enterobacteriaceae, *Legionella,* mixed anaerobes (aspiration), *Pseudomonas aeruginosa*[b] | *Candida albicans*[b] *Aspergillus* species | *Mycoplasma pneumoniae, Pneumocystis carinii,*[b] *Chlamydia trachomatis* (infants), *Chlamydia pneumoniae* |
| Chronic pneumonia | Rare | *Mycobacterium tuberculosis,* other mycobacteria, *Nocardia* | *Coccidioides immitis,*[c] *Blastomyces dermatitidis,*[c] *Histoplasma capsulatum,*[c] *Cryptococcus neoformans* | *Paragonimus westermani*[c] |
| Lung abscess | None | Mixed anaerobes, *Actinomyces, Nocardia, S. aureus,*[d] Enterobacteriaceae,[d] *P. aeruginosa*[b,d] | *Aspergillus* species | *Entamoeba histolytica* |
| Empyema | None | Mixed anaerobes, *S. aureus,*[d] *S. pneumoniae,*[d] Enterobacteriaceae, *P. aeruginosa*[d] | Rare | |

[a] Occurrence limited to seasonal epidemics.

[b] Primarily infects the immunologically compromised host.

[c] Geographically limited.

[d] Infection develops during or after acute pneumonia.

however, may provide the initial predisposition toward bacterial infection. Viruses are extremely rare as a cause of chronic as opposed to acute lower respiratory tract infections, although some symptoms of the acute infection, such as cough, may persist for weeks until the bronchial damage has healed. Influenza virus is noteworthy as a cause of acute life-threatening pneumonia, even in previously healthy young adults. Pneumonia caused by bacteria such as enteric Gram-negative rods, *Pseudomonas,* and *Legionella* is primarily limited to patients with serious debilitating underlying disease or as a complication of hospitalization and its procedures (nosocomial infection). At any age, the pneumococcus is the most common bacterial cause of acute pneumonia, and Gram-negative infections other than *Haemophilus* are rare in children unless they have cystic fibrosis or immunodeficiency. Acute and subacute pneumonia may be due to *Chlamydia; C. trachomatis* is almost exclusively limited to infants less than 7 months of age, whereas *C. pneumoniae* commonly affects school children and young adults, producing both bronchitis and pneumonia.

Gram-negative pneumonias occur in debilitated hosts

Pneumoccoccus is most common cause of acute bacterial pneumonia

Lung abscess and empyema follow infections with the more destructive organisms or aspiration of mixed anaerobic flora from the oropharynx. Several clinical clues can suggest some of the etiologic agents, given a typical clinical syndrome. For example, *Nocardia* and mycobacteria, which are strict aerobes, tend to produce upper lobe infiltrates, whereas aspiration pneumonia caused by anaerobes tends to develop in the most dependent parts of the lung. Textbooks on infectious disease should be consulted for further details regarding these features.

Lung abscess has different patterns

## GENERAL DIAGNOSTIC APPROACHES

The degree of difficulty in establishing an etiologic diagnosis for a lower respiratory tract infection depends on the number of organisms produced in respiratory secretions, whether the causative species is normally found in the oropharyngeal flora, and how easily it is grown. In the presence of typical clinical findings, the isolation of influenza virus from the throat or of *M. tuberculosis* from sputum is sufficient for diagnosis of influenza or tuberculosis, because these organisms are not normally found in such sites. The same cannot be said for *S. pneumoniae* and most bacterial pathogens, because they may be found in the throat in a significant number of healthy persons (see Chapter 9).

Interpretation depends on whether agent is found in normal flora

The examination of expectorated sputum has been the primary means of diagnosing the causes of bacterial pneumonia, but this approach has several advantages and disadvantages. The advantages are ease of collection and absence of risk to the patient. The primary disadvantage is the confusion that results from contamination of the sputum with oropharyngeal flora in the process of expectoration and excessive contamination with saliva. Efforts have been unsuccessful to remove saliva from sputum by washing or to accomplish interpretive differentiation of infective from normal flora by quantitative culture as with urine specimens (see Chapter 66). The quality of a sputum sample can be enhanced by collection early in the morning (just after the patient arises), careful instruction of the patient, and occasionally by the use of saline aerosols (induced sputum) under the supervision of an inhalation therapy specialist. The worst results can be expected when the physician's only involvement is writing an order, which is then passed down the ward chain of command to an orderly, who directs the patient to put his "sputum" in a cup placed at the bedside.

Sputum collection has problems of quality and specificity

Contamination with oropharyngeal secretions is primary problem

Microscopic examination before culture of direct Gram smears of specimens alleged to be sputum has proved useful. Polymorphonuclear leukocytes and large numbers of a single morphologic type of organism are typical findings in sputum from patients with bacterial pneumonia. Squamous epithelial cells from the oropharynx and a mixed bacterial population are characteristic of saliva (Fig 64–1). Unfortunately, most specimens are a mixture of both, which makes interpretation more difficult. Studies have shown that more than 10 to 25 squamous epithelial cells per low-power microscopic field are evidence of excessive salivary contamination, and such specimens should not be cultured because the results may be misleading. Thus, the direct Gram smear is crucial to the use of expectorated sputum for diagnosis of acute bacterial pneumonia. The smear may be useful in the absence of cultural results, but cultures are useless without a Gram smear to assess specimen quality.

Microscopic characteristics of sputum can differentiate from saliva

Salivary specimens should not be cultured

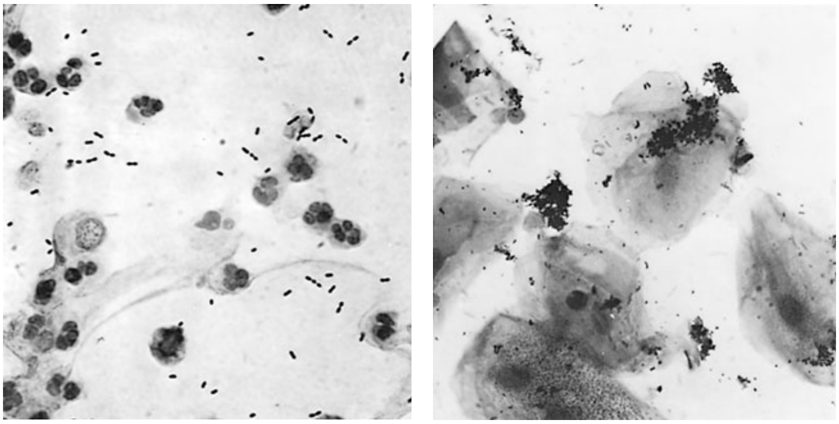


FIGURE 64–1
Comparison of findings in sputum and saliva. True sputum **(A)** should show an abundance of inflammatory cells and no squamous epithelial cells. In acute bacterial pneumonia, large numbers of a single organism are usually present. This Gram smear shows large numbers of polymorphonuclear leukocytes and *Streptococcus pneumoniae.* Saliva **(B)** typically contains squamous epithelial cells and a mixed bacterial population.

Another approach is to attempt a more direct collection from the lung using methods that bypass the oropharyngeal flora. This approach may be used in patients who are not producing sputum or in cases where analysis of expectorated sputum has been inconclusive. The major techniques include transtracheal aspiration, bronchoalveolar lavage (BAL), direct aspiration, and open biopsy. In transtracheal aspiration, an incision is made in the cricothyroid membrane and a catheter advanced deep into the tracheobronchial tree to aspirate sputum directly. This method is useful in diagnosis of both pneumonia and lung abscess. BAL is a modification of bronchoscopy in which the bronchi and alveoli are infused with saline, which is aspirated back through the bronchoscope.

Transtracheal and direct lung aspiration bypass oral flora

Specimens obtained by BAL have been increasingly useful for demonstration of organisms such as *Pneumocystis carinii* that were previously only seen in open lung biopsies. Because BAL involves initial passage of the instrument through the upper airway, interpretation must take into account the possibility of some contamination with oropharyngeal secretions. Aspirates taken through tracheostomies or endotracheal tubes are of almost no value, because these sites become colonized with Gram-negative bacteria within hours of their implantation. Direct aspiration through the chest wall can be used for diagnosis of pneumonia or empyema if the involved area can be well localized and is at the lung periphery. In some cases an open lung biopsy is the only way to obtain diagnostic material. Bacteremia may occur in acute pneumonia, particularly in its early stages. A blood culture should be part of the evaluation of every acute pneumonia. If positive, it can confirm or overrule a diagnosis based on expectorated sputum culture.

BAL washes material from deep in the lung

Blood culture is valuable in acute pneumonia

Once an appropriate specimen is obtained, diagnosis is usually readily made by culture using the methods described in Chapter 15 and in the sections on the individual etiologic agents. Only specimens collected by one of the invasive techniques should be used for anaerobic culture, because expectorated sputum is invariably contaminated with oropharyngeal anaerobes and results are meaningless.

Anaerobic infections cannot be diagnosed from expectorated sputum

## GENERAL PRINCIPLES OF MANAGEMENT

The general principles of management of lower respiratory tract infections are similar to those of middle tract infections. Drainage or surgical measures are needed more often as adjuncts to antimicrobial therapy in cases of chronic pneumonia, lung abscess, and empyema. When bacterial infection is considered, empirical therapy is usually given until the results of cultures and antimicrobial susceptibility tests are available. Treatment may vary from penicillin alone for a previously healthy individual in whom the most reasonable nonviral possibility is *S. pneumoniae,* to multiple drugs for a debilitated or immunocompromised patient, in whom the possibilities are much broader.

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CHAPTER 65

# Enteric Infections and Food Poisoning

KENNETH J. RYAN

Acute infections of the gastrointestinal tract are among the most frequent of all illnesses, exceeded only by respiratory tract infections such as the common cold. Diarrhea is the most common manifestation of these infections; however, because it is usually self-limiting within hours or days, most of those afflicted do not seek medical care. Nonetheless, in the United States, gastrointestinal infection remains one of the three most common syndromes seen by physicians who practice general medicine. Worldwide, diarrheal disease remains one of the most important causes of morbidity and mortality among infants and children. It has been estimated that in Asia, Africa, and Latin America, depending on socioeconomic and nutritional factors, a child's chance of dying of a diarrheal illness before the age of 7 years can be as high as 50%. In developed countries, mortality is very much lower, but it is still significant. This chapter will summarize the known etiologies and epidemiologic circumstances of these infections, as well as diagnostic methods and some aspects of management. Chapters on the individual etiologic agents should be consulted for details.

Diarrheal diseases in developing countries cause death of children

## CLINICAL FEATURES

The most prominent clinical features of gastrointestinal infections are fever, vomiting, abdominal pain, and diarrhea. Their presence varies with different diseases and different stages of infection. The occurrence of diarrhea is a central feature, and its presence and nature form the basis for classification of gastrointestinal infections into three major syndromes: watery diarrhea, dysentery, and enteric fever.

### Watery Diarrhea

The most common form of gastrointestinal infection is the rapid development of frequent intestinal evacuations of a more or less fluid character known as diarrhea (derived from the Greek "dia," for "through," and "rhein," meaning to flow like a stream). Nausea, vomiting, fever, and abdominal pain may also be present, but the dominant feature is intestinal fluid loss. Diarrhea is produced by pathogenic mechanisms that attack the proximal small intestine, the portion of the bowel in which more than 90% of physiologic net fluid absorption occurs. The purest form of watery diarrhea is that produced by enterotoxin-secreting bacteria such as *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC), which cause fluid loss without cellular injury. Other common pathogens that damage the epithelium, such as rotaviruses, also cause fluid loss, but are more likely to cause fever and vomiting as well. Most cases of watery diarrhea run an acute but brief

Fluid loss from proximal small intestine is the primary mechanism

(1 to 3 days) self-limiting course. Exceptions are those caused by *V. cholerae,* which usually produces a more severe illness, and those caused by *Giardia lamblia,* which produces a watery diarrhea that may last for weeks.

## Dysentery

Inflammation, cytotoxins, or invasion produce pus and blood

Colon is primary location

Dysentery begins with the rapid onset of frequent intestinal evacuations, but the stools are of smaller volume than in watery diarrhea and contain blood and pus. If watery diarrhea is the "runs," dysentery is the "squirts." Fever, abdominal pain, cramps, and tenesmus are frequent complaints. Vomiting occurs less often. The focus of pathology is the colon. Organisms causing dysentery can produce inflammatory and/or destructive changes in the colonic mucosa either by direct invasion or by production of cytotoxins. This damage produces the pus and blood seen in the stools but does not result in substantial fluid loss because the absorptive and secretory capacity of the colon is much less than that of the small bowel. Dysenteric infections generally last longer than the common watery diarrheas, but most cases still resolve spontaneously in 2 to 7 days.

## Enteric Fever

Systemic disease begins in the intestine

Focus often becomes lymphoid and reticuloendothelial invasion

Enteric fever is a systemic infection, the origin and focus of which are the gastrointestinal tract. The most prominent features are fever and abdominal pain, which develop gradually over a few days in contrast to the abrupt onset of the other syndromes. Diarrhea is usually present but may be mild and not appear until later in the course of the illness. The pathogenesis of enteric fever is more complex than that of watery diarrhea or dysentery. It generally involves penetration by the organism of the cells of the distal small bowel with subsequent spread outside the bowel to the biliary tract, liver, mesentery, or reticuloendothelial organs. Bacteremia is common, occasionally causing metastatic infection in other organs. Typhoid fever caused by *Salmonella enterica* serotype Typhi is the only infection for which these events have been well studied. Although it is usually self-limiting, enteric fever carries a significant risk of serious disease and significant mortality.

# COMMON ETIOLOGIC AGENTS

Clinical syndromes overlap for specific etiologic agents

Great advances have been made in our understanding of gastrointestinal infections. Before the late 1960s, fewer than 20% of the infectious syndromes described above could be linked to a specific etiologic agent by any known diagnostic method regardless of cost. The organisms listed in Table 65–1 now account for 80 to 90% of cases, although diagnostic methods for all of them are not yet practical for clinical laboratories. The primary clinical syndrome listed for each agent in Table 65–1 should not be regarded as absolute because there are individual variations and overlap; some pathogens cause more than one syndrome. For example, *Shigella* infections frequently go through a brief watery diarrhea stage before localizing in the colon, and *Campylobacter* enteritis usually begins with fever, malaise, and abdominal pain, followed by dysentery. In any single case, the clinical findings may suggest a range of etiologic agents, but none is sufficiently specific to be diagnostic of any single organism.

# EPIDEMIOLOGIC SETTING

Epidemiologic setting narrows the diagnostic possibilities

The epidemiologic setting of the infection is of great importance in assessing the relative probability of the infectious agents. When combined with clinical findings, the differential diagnosis can often be limited to two or three organisms. The major epidemiologic settings are (1) endemic infection, (2) epidemic infection, (3) traveler's diarrhea, (4) food poisoning, and (5) hospital-associated diarrhea.

## Endemic Infections

High frequency in children is related to fecal–oral spread and lack of immunity

By definition, endemic diarrheas are those that occur sporadically in the usual living circumstances of the patient (from the Greek "endemos," dwelling in a place). Some organisms are endemic worldwide, whereas others are geographically limited. There are also seasonal variations and age-related attack rates within the endemic foci. In developed countries the most common causes of endemic gastrointestinal infections are rotaviruses, caliciviruses, *Campylobacter, Salmonella,* and *Shigella.* All are more common in infants and children because they are more prone to fecal–oral spread and because development of immunity is related to age. Rotaviruses account for 40 to 60% of diarrheal infections occurring during the cooler months in infants and children less than 2 years of age but are uncommon in older persons.

The geographically limited agents are common only in the areas listed (see Table 65–1). These distributions are not fixed, making it necessary to keep abreast of geographic changes in the distribution of established agents as well as the recognition of new ones. For example, cholera has long been limited to warm-climate river deltas in Asia, Africa, and the Middle East, but recently it has spread to South and Central America and the Gulf Coast of Louisiana and Texas.

Geographic distributions change

## Epidemic Infections

Under certain epidemiologic conditions some of the organisms responsible for endemic infections can spread beyond the family unit to cause epidemics involving regional, national, and even international populations. The diarrheal diseases most frequently associated with epidemics are typhoid fever, cholera, and shigellosis. For all three, epidemics are related to the failure of basic public health sanitary measures. For example, *Salmonella* serotype Typhi and *Vibrio cholerae* may be spread for some distance through the water supply, a route blocked by modern sewage and water treatment practices. When these procedures are not employed or are interrupted by equipment failure or natural disasters (floods, earthquakes), these diseases can and do recur in epidemic form. Epidemics of shigellosis may be water-borne under the same conditions, but *Shigella* dysentery is more typically a disease "of wars and armies, and of crowds and movement."* The very low infecting dose of *Shigella* can make spreading through direct contact reach epidemic proportions when crowding and poor sanitary facilities are combined. *Giardia, Cryptosporidium,* and *E. coli* O157:H7 were the most frequent identified causes of recent waterborne epidemics in the United States.

Typhoid, cholera, and shigellosis spread where hygiene is poor or after major disasters

Although such epidemics are usually associated with the 19th century, it is clear that the potential remains. In the late 1970s large epidemics of both typhoid fever and shigellosis spread through Central and South America. In 1973 more than 200 cases of typhoid fever in Florida were associated with a defective chlorinator in the local water system. The current cholera epidemic claimed thousands of lives in South America in the last decade of the 20th century.

Most recent epidemic is cholera in South America

## Traveler's Diarrhea

From 20% to 50% of travelers from developed countries who go to less developed countries experience a diarrheal illness in the first week that is usually brief but can be serious. The common names applied to this syndrome, such as "Delhi belly" and "Montezuma's revenge," reflect geographic associations and the cumulative frustration of those forced to spend part of their vacation next to the toilet rather than the swimming pool.

Visits to developing countries are frequently marred

The most extensive studies of traveler's diarrhea have involved travelers from the United States to Latin American countries, particularly Mexico. In nearly 50% of these cases, the diarrhea is caused by enterotoxigenic strains of *E. coli* acquired during travel. *Shigella* infections account for another 10 to 20%, and the remaining cases are attributable to various pathogens or unknown causes. Ingestion of uncooked or incompletely cooked foods is the most likely source of infection, but most epidemiologic studies have not shown specific food associations. An exception is the strong relationship between toxigenic *E. coli* diarrhea and the consumption of salads containing raw vegetables. "Don't drink the water" still seems like sound advice for travelers to countries where hygiene remains poor, but the adage is not well supported by studies relating infection to water or ice consumption.

ETEC is the predominant cause of traveler's diarrhea

Travelers should avoid salads and other uncooked foods

* Christie AB. *Infectious Disease, Epidemiology and Clinical Practice,* 2nd ed. New York: Churchill Livingstone; 1974, p 137.

TABLE 65–1

## Features of Infectious Gastrointestinal Syndromes

| | | | | Laboratory Diagnosis[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Culture | | | Serology | |
| Organism | Common Distribution | Clinical Syndrome | Pathogenic Mechanism | Stool Microscopy | Stool[b] | Blood | Toxin in Stools | Antibody Detection | Antigen Detection |
| *Salmonella* serotypes | Worldwide | Dysentery | Mucosal invasion | PMNs | + | – | – | – | – |
| *Salmonella* serotype typhi | Tropical, developing countries | Enteric fever | Penetration, spread | Monocytes | + | + | – | + | – |
| *Shigella* spp. | Worldwide | Dysentery | Mucosal invasion, cytotoxin | PMNs, RBCs | + | – | – | – | – |
| *Shigella dysenteriae* (Shiga) | Tropical, developing countries | Dysentery | Mucosal invasion, cytotoxin | PMNs, RBCs | + | + | – | – | – |
| *Campylobacter jejuni* | Worldwide | Dysentery | Unknown | PMNs, RBCs | + | – | – | – | – |
| *Escherichia coli* (EIEC) | Worldwide | Dysentery | Mucosal invasion | PMNs, RBCs | +[c] | – | – | – | – |
| *E. coli* (ETEC) | Worldwide[d] | Dysentery | Enterotoxin(s) | – | +[c] | – | – | – | – |
| *E. coli* (EHEC) | Worldwide | Watery diarrhea | Cytotoxin | RBCs | +[c] | – | – | – | – |
| *E. coli* (EPEC) | Worldwide[d] | Watery diarrhea | Adherence | – | +[c] | – | – | – | – |
| *Vibrio cholerae* | Asia, Africa, Middle East, Central and South America, Louisiana, Texas | Watery diarrhea | Enterotoxin | – | + | – | – | – | – |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Vibrio parahaemolyticus* | Seacoast | Watery diarrhea | Unknown | – | + | – | – | – | – |
| *Yersinia enterocolitica* | Worldwide | Enteric fever[c] | Penetration, spread | – | + | + | – | – | – |
| *Clostridium difficile* | Worldwide | Dysentery | Cytotoxin, enterotoxin | – | + | – | + | – | – |
| *Clostridium perfringens* | Worldwide | Watery diarrhea | Enterotoxin | – | + | – | – | – | – |
| *Bacillus cereus* | Worldwide | Watery diarrhea | Enterotoxin | – | + | – | – | – | – |
| Rotavirus | Worldwide | Watery diarrhea | Mucosal destruction | Electron microscopy[f] | – | – | – | – | + |
| Caliciviruses | Worldwide | Watery diarrhea | Mucosal destruction | Electron microscopy[f] | – | – | – | – | – |
| *Giardia lamblia* | Worldwide | Watery diarrhea | Mucosal irritation | Flagellates, cysts | – | – | – | – | – |
| *Entamoeba histolytica* | Worldwide[d] | Dysentery | Mucosal invasion | Amebas, PMNs | – | – | – | + | – |
| *Cryptosporidium* | Worldwide | Watery diarrhea | ?toxin | Acid-fast oocysts | – | – | – | – | – |

*Abbreviations:* RBCs, red blood cells; PMNs, polymorphonuclear leukocytes; EIEC, enteroinvasive *E. coli;* ETEC, enterotoxigenic *E. coli;* EHEC, enterohemorrhagic *E. coli;* EPEC, enteropathogenic *E. coli.*

[a] Positive sign indicates procedure is useful and usually available in clinical laboratories.

[b] Which cultures are done routinely depends on the laboratory and/or physician's request.

[c] Organism may be isolated in culture, but demonstration of pathogenic potential (toxin production, etc.) is limited to specialized laboratories.

[d] Organism is more common in developing countries.

[e] Infection may also manifest watery diarrhea or dysentery.

[f] Appropriate methods may be available in only a limited number of laboratories.

## Food Poisoning

Single-source outbreaks are becoming larger with modern food processing and distribution

Many gastrointestinal infections involve food as a vehicle of transmission. The term "food poisoning," however, is usually reserved for instances in which a single meal can be incriminated as the source. This situation typically arises when multiple cases of the same gastrointestinal syndrome develop at the same time among persons whose only common experience is a meal shared at a social event or restaurant. The probable etiologic agent can usually be assessed from knowledge of the incubation period, the food vehicle, and the clinical findings. Changes in the importation, processing, and distribution of foods have increased the complexity and potential for food-borne transmission of enteric pathogens. Outbreaks that in the past might have been limited, may now be widely distributed by fast-food chains or airline catering services.

Diseases from ingestion of preformed toxin have short incubation periods

The most common causes of food poisoning are shown in Table 65–2. Some are not infections but intoxications, caused by ingestion of a toxin produced by bacteria in the food before it was eaten. Intoxications have shorter incubation periods than infections and may involve extraintestinal symptoms (eg, the neurologic damage in botulism). Infectious food poisoning does not differ from endemic diarrheal infections caused by the same species. The length of the incubation period and the severity of the symptoms are generally related to the number of organisms ingested.

The epidemiologic circumstances of food poisoning vary with the etiologic agent but virtually always involve a breach in the recommended procedures for handling food. The organisms may be present as contaminants in raw food before cooking or introduced by a

TABLE 65–2

**Clinical and Epidemiologic Features of Food Poisoning**

| Etiology | Percentage of Cases[a] | Typical Incubation Period | Primary Clinical Findings | Characteristic Foods |
|---|---|---|---|---|
| **Intoxication**[b] | | | | |
| *Bacillus cereus* (vomiting toxin) | 1–2 | 1–6 h | Vomiting, diarrhea | Rice, meat, vegetables |
| *Clostridium botulinum* | 5–15 | 12–72 h | Neuromuscular paralysis | Improperly preserved vegetables, meat, fish |
| *Staphylococcus aureus* | 5–25 | 2–4 h | Vomiting | Meats, custards, salads |
| Chemical[c] | 20–25 | 0.1–48 h | Variable | Variable |
| **Infections**[d] | | | | |
| *Clostridium perfringens* | 5–15 | 9–15 h | Watery diarrhea | Meat, poultry |
| *Salmonella* | 10–30 | 6–48 h | Dysentery | Poultry, eggs, meat |
| *Shigella* | 2–5 | 12–48 h | Dysentery | Variable |
| *Vibrio parahaemolyticus* | 1–2 | 10–24 h | Watery diarrhea | Shellfish |
| *Trichinella spiralis* | 5–10 | 3–30 days | Fever, myalgia | Meat, especially pork |
| Hepatitis A | 1–3 | 10–45 days | Hepatitis | Shellfish |

[a] Based on documented outbreaks reported to the Centers for Disease Control and Prevention, Atlanta (variable from year to year).

[b] Disease caused by toxin in food at time of ingestion.

[c] Includes heavy metals, monosodium glutamate, mushrooms, and various toxins of nonmicrobial origin.

[d] Disease caused by infection after ingestion.

carrier or contaminated utensil involved in preparation. Causes of bacterial food poisoning include failure to kill the organisms by adequate cooking, almost always followed by a period of warming (incubation) long enough for the organisms to multiply to infectious numbers or, in the case of toxigenic disease, to produce sufficient toxin to cause disease. In 80 to 90% of investigated outbreaks of bacterial food poisoning, the most important contributing factor is the use of improper storage temperatures for the food. This factor may obtain in home-cooked meals as well as those prepared in restaurants, in schools, or at large social events such as community picnics.

Infection is associated with improper cooking and/or storage

The relative frequency of each etiologic agent and the foods most frequently involved are also shown in Table 65–2. This information is based on outbreaks investigated by public health agencies, but it is generally accepted that these represent the "tip of the iceberg" due to underreporting. Large outbreaks, restaurant-associated outbreaks, and outbreaks involving serious illness with hospitalization or death are more likely to be reported to health authorities than are mild diarrheas after a dinner party or airline meal. In recent years, of the 400 to 500 outbreaks (10,000 to 15,000 cases) reported each year in the United States, fewer than 200 are "solved." Food poisoning characterized by a short incubation period (eg *Staphylococcus aureus*) is more likely to be recognized because it can easily be associated with a specific meal and because the food itself may still be available for examination. There are also large geographic differences in reporting. For example, in 1979, New York City, in which 50% of the state population resides, reported 98% of New York state's food-borne outbreaks, and Connecticut reported more outbreaks than all of the southeastern states combined.

Reporting of outbreaks varies greatly

Sampling problems aside, the food poisoning syndromes listed in Table 65–2 are well recognized, with *Salmonella, Clostridium perfringens,* and *S. aureus* accounting for more than 70% of those for which a microbial etiology can be found. For bacterial infections such as *Salmonella* and *Shigella,* which are not normal members of the stool flora, establishing the diagnosis by isolating the causative organism is relatively easy. If the circumstances indicate *C. perfringens* or *S. aureus* food poisoning, investigation involves cultures of vomitus, stool from several cases, and the suspect food. In some cases, toxin detection is required to establish the etiology and source. Such investigations are best coordinated by public health authorities, who can also address the legal and community implications of the outbreak. For example, one investigation of *Salmonella* food poisoning led to the discovery that the owner of a restaurant was keeping and slaughtering chickens at the restaurant. Although this practice may have provided very fresh chicken, it guaranteed *Salmonella* contamination of the entire kitchen.

Determining the cause of microbial food poisoning is best done by public health authorities

## Hospital-associated Diarrhea

The hospital environment should not allow spread of the usual causes of endemic intestinal infection. When such infection occurs, it can usually be traced to an employee who continues working while ill or to contaminated food prepared outside the hospital that is "smuggled" in by the patient's friends. Two special causes of hospital-associated diarrhea are caused by *E. coli* in infants and *Clostridium difficile* in patients treated with antimicrobial agents. Fortunately, *E. coli* outbreaks have become rare. *C. difficile* accounts for more than 90% of cases of a syndrome that ranges from mild diarrhea to fulminant pseudomembranous colitis during or after treatment with antibiotics. The responsible toxigenic *C. difficile* may be resident in the patient's intestinal flora before administration of antimicrobics or be acquired by spread from other patients in the hospital. Rotaviruses can also cause hospital outbreaks in infants.

*E. coli, C. difficile,* and rotaviruses can cause hospital outbreaks

# GENERAL DIAGNOSTIC APPROACHES

Laboratory diagnostic procedures (summarized in Table 65–1) include microscopic examination, culture, toxin detection, and serologic procedures. The relative value of each is different for the various etiologies. The diagnostic approach therefore requires that the physician assess the clinical and epidemiologic features of the case, decide which organisms are potential causes, and provide this assessment to the laboratory so that appropriate procedures will be used.

### Microscopic Examination

Stool microscopy demonstrates white blood cells and parasites

Electron microscopy detects rotaviruses

Microscopic examination is of value in the assessment of bacterial infections when they are positive. The presence of polymorphonuclear leukocytes or blood in the stool correlates with organisms that produce disease by invasion, but false-negative results are common. The leukocytes may be seen in unstained or methylene blue–stained wet mount preparations; the absence of fecal leukocytes, however, does not exclude invasive diarrhea. The observation and morphologic characterization of amebas and flagellates on wet or stained preparations are the primary means by which amebic (*Entamoeba histolytica*) and flagellate (*Giardia lamblia*) infections are diagnosed. Rotaviruses and other viruses of diarrhea cannot be grown in cell culture but can be detected by electron microscopy.

### Culture

Blood cultures are positive in early stages of enteric fever

Stool culture requires selective media for common agents

Isolation of the etiologic agent is the primary means by which bacterial enteric infection is diagnosed. In enteric fever the organism is typically present in the blood in the early stages of disease. Blood cultures are, however, usually negative in watery diarrhea and dysenteric infections, and stool culture must be relied upon for diagnosis. Fortunately, several good selective media have been developed for both direct plating and enrichment culture, which allow isolation of the infecting organism in the presence of a predominant normal flora. Selective media are then used for the various enteric pathogens (see Chapter 15). Media routinely used may vary between clinical laboratories but should include those appropriate for *Salmonella, Shigella,* and *Campylobacter jejuni.* Diarrhea caused by *E. coli* is a special problem, because the methods that define the enterotoxigenic, invasive, or other pathogenic mechanisms are not yet practical for clinical laboratories.

### Toxin Assay

Cell culture or antigen assays detect *C. difficile* toxin

The B cytotoxins of *C. difficile* can be detected by its cytopathic effect in a cell culture system. In most clinical cases, enough toxin is present for direct detection in a stool specimen. This assay is currently available only in reference laboratories. Methods that detect the *C. difficile* A and B toxins by latex agglutination are now in common use. The cost-benefit of various combinations of toxin detection methods is still controversial.

### Antigen and Antibody Detection

Serology is generally ancillary

Antigen detection available for a rotaviruses

At present, antibody detection is useful in the diagnosis of amebic dysentery caused by *E. histolytica* and of typhoid fever. Both are considered ancillary to the primary diagnostic tests, which involve specific detection of the organism by microscopic and cultural methods. Reagents are commercially available for the detection of rotavirus antigen in stool by latex agglutination or enzyme immunoassay. These methods have a sensitivity roughly comparable to that of electron microscopy. Serologic methods have been described for many other causes of gastrointestinal infection but are not generally used because of lack of sensitivity, specificity, or availability of reagents.

## OTHER CAUSES OF INTESTINAL INFECTION

Candidate agents await proof

Despite recent advances in defining the etiologies of enteric infections, there are surely more to be discovered. Organisms not listed in Table 65–1, such as *Aeromonas, Citrobacter,* and *Plesiomonas,* have occasionally been associated with intestinal infections, but the evidence for their enteropathogenicity is not yet strong enough to interpret their isolation from individual cases. At our present state of knowledge, it is not useful to attempt isolation of these organisms unless strong epidemiologic evidence, such as a food-borne outbreak, supports interpretation of the results.

## GENERAL PRINCIPLES OF MANAGEMENT

In most gastrointestinal infections, the primary goal of treatment is relief of symptoms, with particular attention to maintaining fluid and electrolyte balance. The effects of common antidiarrheal medications such as subsalicylate-containing compounds (Pepto-Bismol) or antispasmodics (loperamide) are variable, depending on the etiology. In general, they may be helpful for the watery diarrhea caused by enterotoxins, but not for dysentery caused by mucosal invasion, and antispasmodics may be harmful in the latter instance. Antimicrobial agents are usually not indicated for self-limited watery diarrhea but are required for more severe dysenteric infections. Some enteric infections, such as typhoid fever, are always treated with antimicrobics. Prophylactic regimens for traveler's diarrhea have been effective if it is recognized they do not cover all potential causes. More information on therapy is given in the individual chapters, but texts on infectious diseases should be consulted for specific recommendations.

Maintenance of fluid and electrolyte balance always important

Antimicrobic therapy is primarily for invasive disease

## ADDITIONAL READING

Guerrant RL, et al. Practice guidelines for the management of infectious diarrhea. *Clin Infect Dis* 2001;32:331–350. This consensus statement from the Infectious Disease Society of America gives clear algorithms for diagnosis and management of all the common situations.

Rendi-Wagner P, Kollaritsch H. Drug prophylaxis for travelers' diarrhea. *Clin Infect Dis* 2002;34:628–633. Here is the most recent advice to take along on "spring break."

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CHAPTER 66

# Urinary Tract Infections

KENNETH J. RYAN

Bacterial colonization of the urine within this tract **(bacteriuria)** is common and can, at times, result in microbial invasion of the tissues responsible for the manufacture, transport, and storage of urine. Infection of the upper urinary tract, consisting of the kidney and its pelvis, is known as **pyelonephritis.** Infection of the lower tract may involve the bladder **(cystitis),** urethra **(urethritis),** or prostate **(prostatitis),** the genital organ that surrounds and communicates with the first segment of the male urethra. Because all portions of the urinary tract are joined by a fluid medium, infection at any site may spread to involve other areas of the system.

## EPIDEMIOLOGY

Urinary tract infection (UTI) is among the most common of diseases particularly among women. Prevalence is age and sex dependent. Approximately 1% of children, many of whom demonstrate functional or anatomic abnormalities of the urinary tract, develop infection during the neonatal period. It is estimated that 20% or more of the female population suffers some form of UTI in their lifetime. Infection in the male population remains uncommon through the fifth decade of life, when enlargement of the prostate begins to interfere with emptying of the bladder. In the elderly of both sexes, gynecologic or prostatic surgery, incontinence, instrumentation, and chronic urethral catheterization push UTI rates to 30 to 40%. A single bladder catheterization carries an infectious risk of 1%, and at least 10% of individuals with indwelling catheters become infected.

Young women are commonly infected

Prostate hypertrophy is linked to male disease

## PATHOGENESIS

The urine produced in the kidney and delivered through the renal pelvis and ureters to the urinary bladder is sterile in health. Infection results when bacteria gain access to this environment and are able to persist. Access primarily follows an ascending route for bacteria that are resident or transient members of the perineal flora. These organisms are derived from the large intestinal flora, which is uncomfortably nearby. Conditions that create access are varied, but the most important is sexual intercourse, which has been shown to transiently displace bacteria into the bladder. This puts the female partner is at risk because of the short urethral distance. Other manipulations of the urethra carry risk as well, particularly medical ones such as catheterization. Bacteria may also reach the urinary tract from the bloodstream. This is obviously much less common, because it requires an uncontrolled infection at another site.

Bacteria ascend from perineal flora

Intercourse is common association

Catheters increase risk

Obstruction of urine flow increases risk

Bacterial adherence favors persistence

*E. coli* is virulence model

For bacteria that reach the urinary tract, the major competing forces are the rich nutrient content of the urine itself and the flushing action of bladder voiding. Persistence is favored by host factors that interrupt or retard the urinary flow such as instrumentation, obstruction, or structural abnormalities. In youth, factors are congenital malformations, and with age these include changes that alter the mechanics of outflow, such as prostatic hypertrophy. Bacterial factors include the ability to adhere to the perineal and uroepithelial mucosa and to produce other classical virulence factors like exotoxins. These and other features of pathogenesis are discussed in Chapter 21; *Escherichia coli* is by far the most common and potent UTI pathogen. Urease-producing members of the genus *Proteus* are associated with urinary stones, which themselves are predisposing factors for infection.

## ETIOLOGIC AGENTS

Vast majority due to *E. coli*

Enterobacteriaceae and Gram-positive bacteria appear with complications

Over 95% of UTIs are caused by a single bacterial species, and 90% of these are *E. coli.* Other Enterobacteriaceae, *Pseudomonas,* and Gram-positive bacteria become increasingly frequent with chronic, complicated, and hospitalized patients. Of the Gram-positive bacteria enterococci are the most important. *Staphylococcus saprophyticus,* a coagulase-negative staphylococcus, is now recognized as the etiology in a significant minority of symptomatic infections in young, sexually active women. Yeasts, particularly species of *Candida,* may be isolated from catheterized patients receiving antibacterial therapy and from diabetic individuals, but they seldom produce symptomatic disease.

## MANIFESTATIONS

Some cases are asymptomatic

The clinical manifestations of UTI are variable. Approximately 50% of infections do not produce recognizable illness and are discovered incidentally during a general medical examination. Infections in infants produce symptoms of a nonspecific nature, including fever, vomiting, and failure to thrive. Manifestations in older children and adults, when present, often suggest the diagnosis and sometimes the localization of the infection within the urinary tract.

### Cystitis

Urethral irritation differs from genital infections

Fever is usually absent

The symptoms of cystitis are **dysuria** (painful urination), **frequency** (frequent voiding), and **urgency** (an imperative "call to toilet"). These findings are similar to those of urethritis caused by sexually transmitted agents. The cystitis complex is, in fact, produced by irritation of the mucosal surface of the urethra as well as the bladder. It is clinically distinguished from pure urethritis by a more acute onset, more severe symptoms, the presence of bacteriuria, and in approximately 50% of cases, hematuria. The urine is often cloudy and malodorous and occasionally frankly bloody. Cystitis patients also experience pain and tenderness in the suprapubic area. Fever and systemic manifestations of illness are usually absent unless the infection spreads to involve the kidney.

### Pyelonephritis

Fever and flank pain mark upper tract disease

Prematurity is risk in pregnancy

Chronic pyelonephritis is not linked to UTI

The typical presentation of upper urinary infection consists of **flank pain** and **fever** that exceeds 38.3°C. These findings may be preceded or accompanied by manifestations of cystitis. Rigors, vomiting, diarrhea, and tachycardia are present in more severely ill patients. Physical examination reveals tenderness over the costovertebral areas of the back and, occasionally, evidence of septic shock. In the absence of obstruction, the clinical manifestations usually abate within a few days, leaving the kidneys functionally intact. It has been estimated, however, that 20 to 50% of pregnant women with acute pyelonephritis give birth to premature infants, one of the most serious consequences of UTI. In the presence of obstruction, a neurogenic bladder, or vesicoureteral reflux, clinical manifestations are more persistent, occasionally leading to necrosis of the renal papillae and progressive impairment of kidney function with chronic bacteriuria. If a renal calculus or necrotic renal papilla impacts in the ureter, severe flank pain with radiation to the groin occurs. The term chronic pyelonephritis is used to describe inflamed, scarred, contracted kidneys often in association with compromised renal function. There is no known connection between UTI and chronic pyelonephritis.

## Prostatitis

Infection of the prostate is typically manifested as pain in the lower back, perirectal area, and testicles. In acute infection, the pain may be severe and accompanied by high fever, chills, and the signs and symptoms of cystitis. Inflammatory swelling can lead to obstruction of the neighboring urethra and urinary retention. On rectal palpation, the prostate is boggy and exquisitely tender. Response to antibiotic therapy is good, but occasionally abscess formation, epididymitis, and seminal vesiculitis or chronic infection develop. Typically, acute prostatitis develops in young adults; however, it can also follow placement of an indwelling catheter in an older man. Patients with chronic prostatitis seldom give a history of an acute episode. Many are totally without symptoms; others experience low-grade pain and dysuria. Periodic spread of prostatic organisms to the urine in the bladder produces recurrent bouts of cystitis. In fact, chronic prostatitis is probably the major cause of recurrent bacteriuria in men. The etiologic agents are the same as in cystitis and pyelonephritis.

Back and perirectal pain are signs

Chronic disease a source for cystitis

# DIAGNOSIS

## Specimen Collection

The diagnosis of UTI is based on examination of the normally sterile urine for evidence of bacteria or an accompanying inflammatory reaction. Critical to this examination is the use of appropriate techniques for specimen collection. Urine is most easily obtained by spontaneous micturition. Unfortunately, voided urine is invariably contaminated with urethral flora and, in female patients, perineal and vaginal flora, which can confound the results of laboratory testing. Although the contaminants can never be completely eliminated, their quantity may be diminished by carefully cleansing the periurethrum before voiding and allowing the initial part of the stream to flush the urethra before collecting a specimen for examination. This **clean-voided midstream urine** collection procedure is preferred to catheterization for routine purposes because it avoids the risk of introducing organisms into the bladder. When the laboratory examination of such a specimen produces equivocal results or the patient cannot comply with the requirements of the clean-voided technique, catheterization or suprapubic aspiration from the distended bladder may be necessary.

Midstream collection intended to bypass contamination

Direct collections are confirmatory

For the diagnosis of prostatitis, urine is collected in three segments by interrupting a single bladder excavation. The first voiding is considered a urethral washout. The midstream specimen that follows is used to assess cystitis. The prostate is then massaged, and the final urine is a prostatic secretions washout. The quantitative culture results are then compared. In prostatitis, it is expected that the third specimen contains the largest numbers of the pathogen.

Prostatitis requires three-component voiding

## Microscopic Examination

Approximately 90% of patients with acute symptomatic UTI have pyuria (that is, >10 white cells/$mm^3$ of urine). This finding is also common, however, in a number of noninfectious diseases. More specific is the presence of white cell casts, which occur primarily in patients with acute pyelonephritis. A more sensitive and specific microscopic procedure is a Gram-stained smear of uncentrifuged urine (Fig 66–1). The presence of at least one organism per oil-immersion field is almost always indicative of bacterial infection. The absence of white cells and bacteria in several fields makes the diagnosis of UTI unlikely. However, this finding does not rule it out, especially in young women with acute, symptomatic infection who may be infected with smaller numbers of organisms.

Pyruia suggests UTI but is not specific

Bacteria on unspun smear correlates with bacteriuria

## Chemical Screening Tests

A number of nonmicroscopic urinary screening tests have been commercially marketed within the past several years. The most successful detects leukocyte esterase

Leukocyte esterase detects pyuria

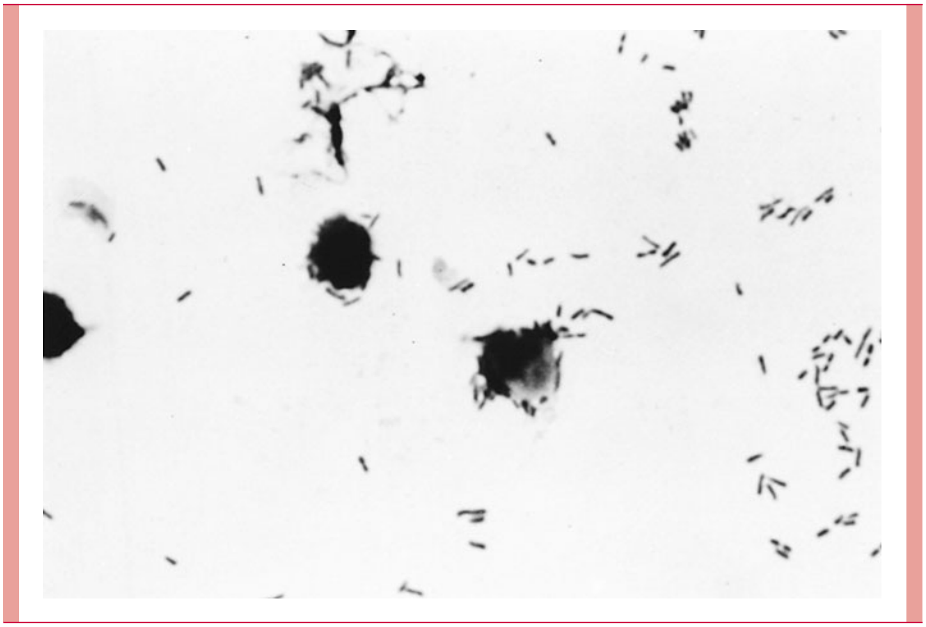

FIGURE 66–1
Gram stain of an uncentrifuged clean voided urine specimen from a patient with an acute *Escherichia coli* urinary tract infection. Some degenerating polymorphonuclear leukocytes and numerous Gram-negative rods are present.

from inflammatory cells and nitrite produced from urinary nitrates by bacterial metabolism. Although technically simpler, the sensitivity and specificity of these products are similar to that of microscopic examination. Like microscopic examination, they do not reliably detect bacteriuria below the level of $10^5$ organisms/mL.

## Urine Culture

$>10^5$ bacteria/mL is typical for UTI

Contaminants can be $>10^5$ bacteria/mL

Based on studies done half a century ago demonstrating that the number of bacteria in infected urine is large, quantitative bacteriology has been the gold diagnostic standard for UTI. Perhaps no number in medicine is better known or more slavishly adhered to than $10^5$ bacteria/mL of urine. Above it is UTI, below it is contamination. We now know that it is possible to void more than $10^5$ of contaminants and to have a genuine UTI with less than $10^5$ bacteria as illustrated in Figure 66–2. Virtually no woman with sterile bladder

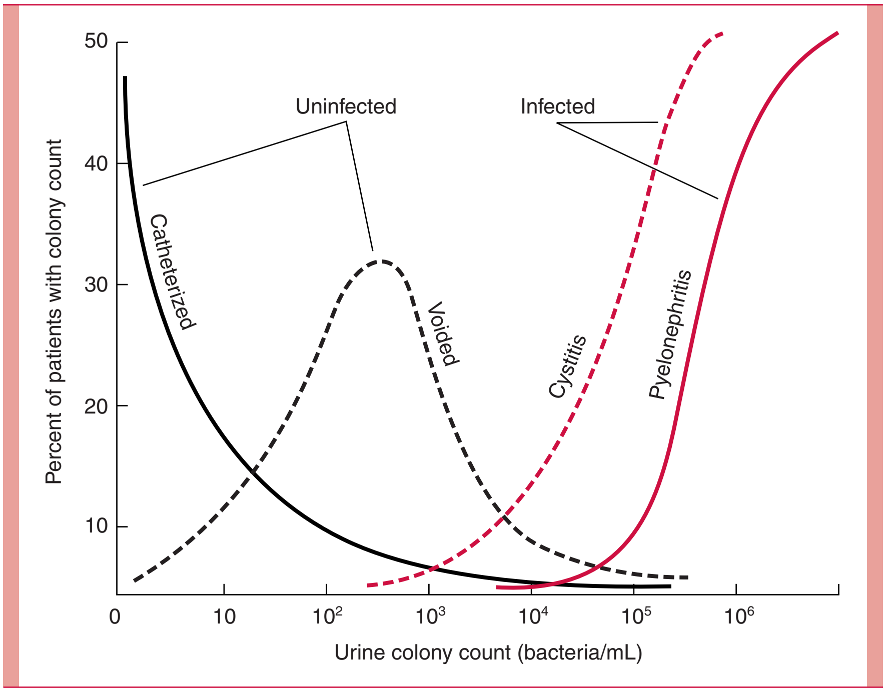


FIGURE 66–2
Quantitative urine culture. Bacteria are routinely quantitated in the range of 10 to $>10^5$. Uninfected persons may show bacteria in the urine due to contamination from the perineal flora. The number are small if the specimen is collected by catheterization but voided (midstream method) specimens contain larger numbers. Patients with pyelonephritis have very high numbers of bacteria but those with only cystitis often have numbers less than $10^5$.

urine, as determined by suprapubic aspiration, can void a sterile specimen even with periurethral cleansing. Voided contaminants are most often mixtures of vaginal flora not associated with UTI such as lactobacilli, diphtheroids, and streptococci, but can include urinary pathogens. Conversely, we now know that bacterial counts in UTI represent a spectrum from $10^2$ to more than $10^6$ bacteria/mL. The lower counts are typical for simple cystitis and the high counts for pyelonephritis. Fully one third of women with UTI limited to the bladder demonstrate counts less than $10^5$ bacteria/mL.

Bacterial counts may be $<10^5$ bacteria/mL in UTIs

Given the overlap, application of these findings to clinical practice requires linking the epidemiologic probability to the clinical findings. If a woman has symptoms of cystitis and a culture positive for a urinary pathogen, the probability she has a UTI is 90%, even if the count is as low as $10^3$ bacteria/mL. If the woman is asymptomatic, the probability drops to 80% even if the count is more than $10^5$/mL. In the latter case, the culture must be repeated before concluding that a UTI is present. Voiding more than $10^5$ of the same contaminant twice in a row is unlikely. There is no reason to repeat positive cultures from symptomatic patients. Catheterized and suprapubic specimens may be accepted at face value, because they come directly from the bladder.

Presence of both pathogens and symptoms is diagnostic

Asymptomatic positives should be repeated

## TREATMENT

The treatment of UTI is best guided by the results of cultures and antimicrobial susceptibility tests. In simple isolated instances of cystitis in a young woman, the etiology is often assumed to be *E. coli* and the antimicrobic selected empirically based on knowledge of the susceptibility of local strains. Sulfonamides and trimethoprim alone or in combination with sulfamethoxazole, a fluoroquinolone, and nitrofurantoin are the agents most commonly used. In most areas, the use of ampicillin is precluded by resistance rates exceeding 25%. For children and patients with risk factors or recurrent infections, empiric therapy should always be confirmed by culture and susceptibility testing. Likewise, the duration of therapy depends on the severity of the infection and the risk status of the patient. Success of treatment may be tested by a follow-up urine culture 1 to 2 weeks after therapy is completed.

Empiric treatment is common

Resistance limits ampicillin use

## PREVENTION

Those with several symptomatic episodes annually may be helped with long-term, low-dose chemoprophylaxis. In women whose recurrences are related to sexual activity, administration of the chemoprophylactic agent may be limited to immediately after intercourse. Infected children, men, and those who experience UTI relapse should be investigated with intravenous pyelography to allow detection and correction of any factor causing predisposition to infection.

Chemoprophylaxis may be effective

## ADDITIONAL READING

Kass EH. Asymptomatic infections of the urinary tract. *Trans Assoc Am Physicians* 1956;69:56–64. This paper, where the $10^5$ bacteria/ mL value comes from, is one of the most cited papers in the medical literature. Many are not aware that the clear separation between UTIs and controls owes much to the fact that the specimens obtained in this study were catheterized, not voided.

Warren JW, Abrutyn E, Hebel JR, et al. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infectious Diseases Society of America (IDSA). *Clin Infect Dis* 1999;29:745–758. This set of guidelines from the IDSA uses an evidence-based approach to the treatment of UTI.

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CHAPTER 67

# Central Nervous System Infections

C. George Ray

The cerebrum, cerebellum, brainstem, spinal cord, and their covering membranes (meninges) constitute the central nervous system (CNS). Because of the unique anatomic and physiologic features of the CNS, infections of this site can represent special challenges to the microbiologist and clinician. The CNS is encased in a rigid, bony vault, and it is highly vulnerable to the effects of inflammation and edema: its critical life-regulatory functions and the metabolic requirements to sustain these functions can also be easily disrupted by infection, with resultant local acidosis, hypoxia, and destruction of nerve cells. Thus, the effects of increased pressure, biochemical abnormalities, and tissue necrosis can be profound and sometimes irreversible. One specialized defense mechanism of the CNS is the blood–brain barrier, which serves to minimize passage of infectious agents and potentially toxic metabolites into the cerebrospinal fluid (CSF) and tissues, as well as to regulate the rate of transport of plasma proteins, glucose, and electrolytes. When CNS infection develops, however, this barrier also poses difficulties in control; some antimicrobial agents and host immune factors, such as immunoglobulins and complement, do not pass as readily from the blood to the site of infection as they do to other tissues.

Blood–brain barrier affects access of microbes, immune factors, and antimicrobics

Within the brain are the ventricles, which are cavities in which CSF is actively produced, primarily by specialized structures called the choroid plexuses. The CSF fills the lateral ventricles in each half of the brain, circulates into a central third ventricle, and then passes through the cerebral aqueduct to emerge through foramina at the brainstem. From cisterns at the base of the brain, the CSF circulates in the subarachnoid space over the entire CNS, including the spinal cord, to supply nutrients and serve as a hydraulic cushion for these tissues. It is reabsorbed primarily by the major venous system in the meninges. Obstruction of the normal flow of CSF in either the internal (ventricular) or external (subarachnoid) systems can result in increased intracranial pressure, because production of CSF by the choroid plexuses will continue within the ventricles. Such impairment of flow or normal reabsorption can occur as a result of inflammation or subsequent fibrosis, leading to dilatation of the ventricles, compression of brain tissue, and a condition known as **hydrocephalus.**

CSF continuously produced by choroid plexus

Obstruction of CSF flow or reabsorption causes hydrocephalus

## ROUTES OF INFECTION

Most CNS infections appear to result from blood-borne spread; for example, bacteremia or viremia resulting from infection of tissue at a site remote from the CNS may result in penetration of the blood–brain barrier. Examples of infectious agents that commonly infect the CNS by this route are *Haemophilus influenzae, Neisseria meningitidis, Streptococcus* *pneumoniae, Mycobacterium tuberculosis,* and viruses such as enteroviruses and mumps (Tables 67–1 and 67–2). The initial source of infection leading to bloodstream invasion may be occult (eg, infection of reticuloendothelial tissues) or overt (eg, pneumonia, pharyngitis, skin abscess or cellulitis, or bacterial endocarditis). Occasionally, the route of infection is from a focus close to or contiguous with the CNS. These possible sources include middle ear infection (otitis media), mastoiditis, sinusitis, or pyogenic infections of the skin or bone. Infection may extend directly into the CNS, indirectly via venous pathways, or in the sheaths of cranial and spinal nerves.

Blood-borne spread most common access to CNS

Direct spread occurs from adjacent infected focus such as middle ear

In some cases, a contiguous or distant infectious focus may not be necessary to produce CNS infection. If an anatomic defect exists in the structures encasing the CNS, infectious agents may readily gain access to the vulnerable site and establish themselves. Such defects may be traumatically or surgically induced or result from congenital malformations. For example, fractures of the base of the skull may produce an opening between the CNS and the sinuses, nasal passages (defects in the cribriform plate), mastoid, or middle ear. All of these sites are contiguous with the upper respiratory tract, which enables a potentially pathogenic member of the respiratory flora to gain ready access to the CNS. Neurosurgical procedures also create transient communications between the external environment and the CNS that can be readily contaminated. This risk can be compounded when foreign bodies, such as shunts or external drainage tubes, must be left in place for the treatment of hydrocephalus. These foreign bodies, when colonized, can serve as chronic foci of infection. Congenital defects, such as meningomyeloceles or sinus tracts through the cranium or spine, may also be sources. The latter may be overlooked; the orifice of the sinus may be a small cleft on the skin surface, or occasionally it may open internally into the intestinal tract. Recurrent purulent meningitis or unusual pathogens in an otherwise healthy host should prompt a careful search for such defects.

Traumatic, surgical, or congenital lesions may give direct access

Implanted foreign bodies such as shunts increase risk

Perhaps the least common route of CNS infection is by intraneural pathways. Agents capable of intraneural spread to the CNS include rabies virus (presumably along peripheral sensory nerves), herpes simplex virus (often, but not exclusively, via the trigeminal nerve root or sacral nerves), polioviruses, and perhaps some togaviruses.

Intraneural pathways operate with a few viruses

Abscesses of the CNS deserve special mention. Although relatively uncommon compared with other CNS infections, they represent a special microbiologic and clinical problem. Such abscesses may be within the tissues of the CNS (eg, brain abscess; Fig 67–1) or localized in the subdural or epidural spaces. They sometimes develop as a complication

TABLE 67–1

### Common Causes of Purulent Central Nervous System Infections

| AGE GROUP | AGENT |
|---|---|
| Newborns (<1 mo old) | Group B streptococci (most common), *Escherichia coli, Listeria monocytogenes, Klebsiella* species, other enteric Gram-negative bacteria |
| Infants and children | *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae* |
| Adults | *S. pneumoniae, Neisseria meningitidis* |
| **SPECIAL CIRCUMSTANCES** | |
| Meningitis or intracranial abscesses associated with trauma, neurosurgery, or intracranial foreign bodies | *Staphylococcus aureus, Staphylococcus epidermidis, S. pneumoniae;* anaerobic Gram-negative and Gram-positive bacteria; *Pseudomonas* species |
| Intracranial abscesses not associated with trauma or surgery | Microaerophilic or anaerobic streptococci, anaerobic Gram-negative bacteria (often mixed aerobic and anaerobic flora of upper respiratory tract origin) |

TABLE 67–2

**Primary Acute Viral Infections of the Central Nervous System**

| Agent | Major Age Group Affected | Seasonal Predominance |
|---|---|---|
| Enteroviruses | Infants, children | Summer–fall |
| Mumps | Children | Winter–spring |
| Herpes simplex | | |
| Type 1 | Adults | None |
| Type 2 | Neonates, young adults | None |
| Arboviruses | | |
| Western equine encephalitis | Infants, children | Summer–fall |
| St. Louis encephalitis | Adults >40 years | Summer–fall |
| California encephalitis | School-aged children | Summer–fall |
| Eastern equine encephalitis | Infants, children | Summer–fall |
| West Nile encephalitis | Adults | Summer–fall |
| Rabies | All ages | Summer–fall |
| Measles | Infants, children | Spring |
| Varicella–zoster | Infants, children | Spring |
| Lymphocytic choriomeningitis | Adults, children | None |
| Epstein–Barr virus | Children, young adults | None |
| Other (eg, myxoviruses, human immunodeficiency virus, cytomegaloviruses) | All ages | Variable |

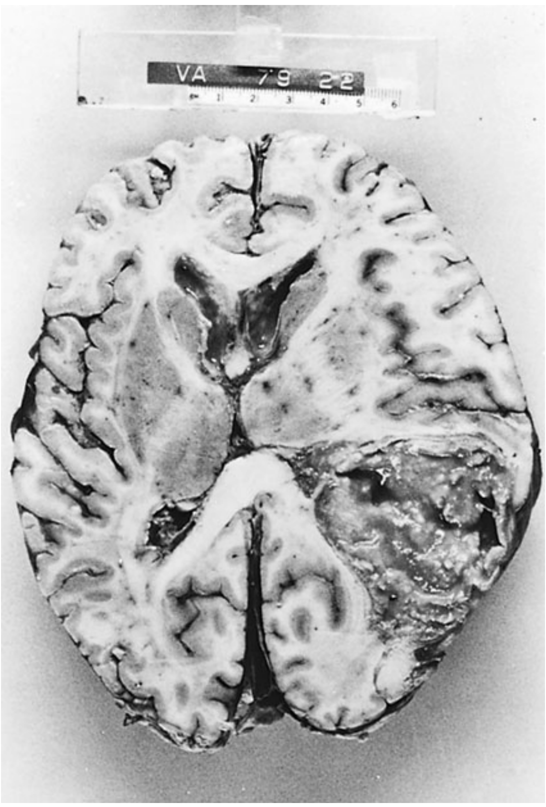


FIGURE 67–1
Coronal section of a brain demonstrating a poorly encapsulated abscess.

Abscesses present diagnostic problems and may localize in brain, at subdural or epidural sites

of pyogenic meningitis. More commonly, abscesses of the CNS result from embolization of bacteria or fungi from a distant focus, such as endocarditis or pyogenic lung abscess; extension from a contiguous focus of infection (eg, sinusitis or mastoiditis); or a complication of surgery or nonsurgical trauma.

## CLINICAL FEATURES

Acute onset and progression with stiff neck and neurologic dysfunction

Usually fatal if untreated

Several terms commonly applied to CNS infections need to be understood. **Purulent meningitis** refers to infections of the meninges associated with a marked, acute inflammatory exudate and is usually caused by a bacterial infection. Such infections frequently involve the underlying CNS tissue to a variable degree, and often the ventricular system is also involved (ventriculitis). Most cases of purulent meningitis are acute in onset and progression and are characterized by fever, stiff neck, irritability, and varying degrees of neurologic dysfunction that, if untreated, usually progress to a fatal outcome. Large numbers of polymorphonuclear leukocytes are present in the CSF of established cases.

Granulomatous infections are chronic

**Chronic meningitis** has a more insidious onset, with progression of signs and symptoms over a period of weeks. This is usually caused by mycobacteria or fungi that produce granulomatous inflammatory changes, but occasionally protozoal agents are responsible (Table 67–3). The cellular response in the CSF reflects the chronic inflammatory nature of the disease.

Aseptic meningitis is commonly of viral etiology

Other causes include syphilis

**Aseptic meningitis** is a term used to describe a syndrome of meningeal inflammation associated mostly with an increase of cells (pleocytosis), primarily lymphocytes and other mononuclear cells in the CSF, and absence of readily cultivable bacteria or fungi. It is associated most commonly with viral infections and is often self-limiting. The syndrome can also occur in syphilis and some other spirochetal diseases, as a response to the presence of drugs or radiopaque substances in the CSF, or from tumors or bleeding involving the meninges or subarachnoid space. The primary site of inflammation is in the meninges without clinical evidence of involvement of the neural tissue. Such patients may have fever, headache, a stiff neck or back, nausea, and vomiting.

**Encephalitis** also implies a primary viral etiology; however, acute or chronic demyelinating diseases with or without inflammation must also be considered. This latter

TABLE 67–3

**Other Causes of Central Nervous System Infections**

| Disease | Agent |
|---|---|
| Chronic granulomatous infection | *Mycobacterium tuberculosis*[a] |
| | *Coccidioides immitis* |
| | *Cryptococcus neoformans* |
| | *Histoplasma capsulatum* |
| Parasitic infection | |
| Protozoa | *Toxoplasma gondii*[b] |
| | *Trypanosoma* |
| | *Acanthamoeba* species |
| Nematodes | *Toxocara* species |
| | *Trichinella spiralis* |
| | *Angiostrongylus cantonensis* |
| Cestodes | *Taenia solium* (cysticercosis) |
| Other | *Leptospira* species |
| | *Treponema pallidum* |
| | *Borrelia burgdorferi* |

[a] Tuberculous meningitis can appear as acute or chronically progressive disease.

[b] Toxoplasmosis of the central nervous system is usually seen in congenital infections or immunocompromised hosts.

group includes the postinfectious or allergic encephalomyelitis syndromes, in which the etiology and pathogenesis are not always clearly defined. Clinically, the diagnosis of encephalitis is applied to patients who may or may not show signs and CSF findings compatible with aseptic meningitis but also show objective evidence of CNS dysfunction (eg, seizures, paralysis, and disordered mentation). Many clinicians use the term **meningoencephalitis** to describe patients with both meningeal and encephalitic manifestations.

Viral and postinfectious etiology most common

**Poliomyelitis** refers to the selective destruction of anterior motor horn cells in the spinal cord and/or brainstem, which leads to weakness or paralysis of muscle groups and occasionally respiratory insufficiency. It is usually associated with aseptic meningitis, sometimes with encephalitis. The polioviruses are the major causes of this syndrome, although coxsackieviruses (primarily type A7) and other enteroviruses, such as enterovirus 71, have been implicated. The hallmark of poliomyelitis is asymmetric flaccid paralysis.

Viral destruction of anterior horn cells causes paralysis

Two other nervous system syndromes presumably associated with infection deserve brief mention. **Acute polyneuritis,** an inflammatory disease of the peripheral nervous system, is characterized by symmetric flaccid paralysis of muscles. In most cases, no specific etiology is found; some, however, have been associated with *Corynebacterium diphtheriae* toxin and infections by bacterial enteric pathogens, cytomegalovirus or Epstein–Barr virus. **Reye's syndrome** (encephalopathy with fatty infiltration of the viscera) is an acute, noninflammatory process, usually observed in childhood, in which cerebral edema, hepatic dysfunction, and hyperammonemia develop within 2 to 12 days after onset of a systemic viral infection. Although the influenza A and B and varicella–zoster viruses have been most frequently implicated in this syndrome, the precise pathogenesis is not yet known. Concomitant salicylate therapy is known to be a contributory factor.

Acute polyneuritis involves peripheral nervous system

Reye's syndrome precipitated by salicylate treatment of systemic viral infection

## COMMON ETIOLOGIC AGENTS

The causes of CNS infections are numerous, as illustrated in Tables 67–1 through 67–3. Acute purulent meningitis is usually caused by one of three organisms: *H. influenzae* type b, *N. meningitidis,* or *S. pneumoniae*. The incidence of *H. influenzae* meningitis has now fallen sharply in the United States as a result of routine immunization. In neonatal infections, group B streptococci or *Escherichia coli* are most frequently implicated. However, many other bacteria can occasionally cause the disease if they gain access to the meninges.

Acute purulent meningitis caused by encapsulated pathogens

Of the viral causes of acute CNS disease, the categories most commonly encountered are the enteroviruses, human immunodeficiency virus, herpes simplex, Epstein–Barr virus, and arthropod-borne viruses. In the United States, enteroviruses account for the greatest proportion of infections. Viral CNS infections can be manifested clinically as aseptic meningitis, encephalitis, or poliomyelitis. The age of the patient and the season of occurrence help somewhat in predicting some of the agents that may be involved, (see Table 67–2); other epidemiologic, ecologic, and clinical factors associated with these infections are discussed in the individual chapters on specific virus groups.

Acute viral disease has a variety of manifestations

Seasonality and patient age important clues

Slow viral infections of the CNS, such as subacute sclerosing panencephalitis (due to measles or sometimes congenitally acquired rubella virus), acquired immunodeficiency syndrome encephalopathy, progressive multifocal leukoencephalopathy (due to JC polyomavirus), and Creutzfeldt–Jacob disease ("unconventional" viruses), are discussed in Chapters 34, 42, 43, and 44, respectively. Other important causes of CNS infections (see Table 67–3) that must not be overlooked include *Mycobacterium tuberculosis* and the deep mycoses (especially *Cryptococcus neoformans* and *Coccidioides immitis*). These chronic infections can be insidious in onset and mimic other processes, thus delaying consideration of the proper diagnosis.

Chronic meningitis is caused by slow-growing agents

Finally, there are noninfectious causes of CNS disease to be considered in the differential diagnosis. These include (1) metabolic disturbances, such as hypoglycemia, diabetic coma, and hepatic failure; (2) toxic conditions, such as those caused by bacterial toxins (diphtheria, tetanus, botulism), insect toxins (tick paralysis), poisons (lead), and drug abuse; (3) mass lesions, such as acute trauma, hematoma, and tumor; (4) vascular lesions, such as intracranial embolus, aneurysm, and subarachnoid hemorrhage; and (5) acute psychiatric episodes.

Noninfectious diseases may mimic infections

## GENERAL DIAGNOSTIC APPROACHES

Except in unusual circumstances, in which severe increases in intracranial pressure make the procedure dangerous, a lumbar puncture is the first step in the workup of a patient with suspected CNS infection. The CSF pressure is determined at the time of the procedure, and CSF is removed for analysis of cells, protein, and glucose. Ideally, the glucose content of the peripheral blood is determined simultaneously for comparison with that in the CSF. Table 67–4 presents guidelines for interpretation of results of CSF analysis; these guidelines represent generalizations, however, and must not be considered as absolute findings in all cases. For example, although a patient with bacterial, mycobacterial, or fungal meningitis usually has a glucose level in the CSF of less than 40 mg/dL, or less than half the blood glucose level (hypoglycorrhachia), this finding may not be present in the early stages of infection. Viral infections of the CNS can occasionally produce low glucose values in the CSF; in addition, the early stages of viral infection may be associated with a preponderance of polymorphonuclear leukocytes. It is clearly important to recognize that viral CNS infections can exist with a negligible CSF cell count. This sometimes also occurs in the early stages of bacterial meningitis.

Lumbar puncture for pressure, cells, protein, and glucose in CSF

CSF glucose should be compared with simultaneous blood level

Realizing the limitations, it is possible to make some general interpretations that are helpful in the diagnosis. Viral CNS infections are usually associated with a preponderance of lymphocytes, a normal glucose value, and a normal or moderately elevated protein level in the CSF. In contrast, acute bacterial meningitis usually causes a CSF pleocytosis consisting primarily of polymorphonuclear cells, a low glucose value, and a high protein level. Mycobacterial and fungal infections are more commonly associated with lymphocytosis (and sometimes moderate eosinophilia) in the CSF; like the acute bacterial infections, however, they tend to lower glucose and increase protein levels markedly.

Viral and granulomatous infections often cause predominance of lymphocytes in CSF

Normal values for CSF are also shown in Table 67–4. Polymorphonuclear cells are not usually seen in normal CSF, but as many as five lymphocytes/mm$^3$ may be found in healthy individuals. Neonatal CSF is considerably more difficult to interpret, because cell counts are often elevated in the absence of infection; glucose values, however, should be within the normal range.

Polymorphonuclear cells not often found in normal CSF

The other major procedures that must be performed on all CSF samples in which any infection is suspected include bacterial cultures and Gram staining. If the CSF is grossly purulent and the patient untreated, a Gram stain of the uncentrifuged CSF or of its centrifuged sediment frequently shows the infecting organism and indicates the specific diagnosis. According to the clinical indications and results of CSF cytology and chemistry, other microbiologic tests may be used, including viral cultures, special stains and cultures for fungi and mycobacteria, immunologic methods to detect fungal or bacterial antigens

Direct staining and culture are definitive diagnostic methods

Tests for free antigens useful in some circumstances

TABLE 67–4

**Findings of Cerebrospinal Fluid Analysis: Normal Versus Infection**

| Clinical Situation | Leukocytes/mm$^3$ | % Polymorphouclears | Glucose (% of blood) | Protein (mg/dL) |
|---|---|---|---|---|
| **Children and Adults** | | | | |
| Normal | 0–5 | 0 | ≥60 | ≤30 |
| Viral infection | 2–2000 (80)[a] | ≤50 | ≥60 | 30–80 |
| Pyogenic bacterial infection | 5–5000 (800) | ≥60 | ≤45[b] | >60 |
| Tuberculosis and mycoses | 5–2000 (100) | ≤50 | ≤45 | >60 |
| **Neonates** | | | | |
| Normal (term) | 0–32 (8) | ≤60 | ≥60 | 20–170 (90) |
| Normal (preterm) | 0–29 (9) | ≤60 | ≥60 | 65–150 (115) |

*a* Numbers in parentheses represent mean values.

*b* Usually very low.

(eg, latex agglutination for *Cryptococcus*), and polymerase chain reactions to detect viral or bacterial nucleic acids.

Tests on specimens other than CSF are selected on the basis of the clinical diagnostic possibilities. If acute bacterial meningitis is suspected, blood cultures should also be used to ensure the diagnosis. Viral cultures of the pharynx, stool, or rectal swabs may provide indirect evidence of CNS infection. In encephalitis, a biopsy specimen of the brain is sometimes obtained for culture, histology, and to demonstrate viral antigen or nucleic acid. Other studies may include acute and convalescent sera for viral serology and serologic tests to detect antibodies to certain fungi, such as *C. immitis*.

Culture of blood and other sites depend on suspected etiology

Biopsy and serology useful for some agents

Intracranial abscesses can often be detected with radiologic techniques, such as computerized tomography or magnetic resonance imaging. A definitive etiologic diagnosis is established by careful aerobic and anaerobic culture of the contents of the abscess.

Imaging methods useful to detect abscesses

## GENERAL PRINCIPLES OF MANAGEMENT

In bacterial, mycobacterial, and fungal infections of the CNS, prompt and aggressive antimicrobial therapy is required. The duration of treatment varies from as little as 10 days for uncomplicated bacterial meningitis, to 12 months or longer for tuberculous meningitis, and to several years for some cases of fungal meningitis.

Antimicrobial therapy is administered immediately

In addition to antimicrobial therapy, correction of associated metabolic defects (acidosis, hypoxia, saline depletion, inappropriate antidiuretic hormone secretion) is necessary. Increased intracranial pressure as a result of vasogenic edema or hydrocephalus must be monitored and controlled accordingly; osmotic agents such as intravenous mannitol are often used to control acute cerebral edema, and neurosurgical shunting procedures may be needed to treat progressive hydrocephalus. Abscesses often require drainage. Except for those patients with herpes simplex encephalitis, who often respond to early treatment with antiviral agents, most viral infections of the CNS can only be managed supportively. This includes specific attention to the metabolic and respiratory problems that may develop in severe cases.

Correction of metabolic defects and raised intracranial pressure important

Viral infections are managed supportively

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CHAPTER 68

# Intravascular Infections, Bacteremia, and Endotoxemia

C. GEORGE RAY AND KENNETH J. RYAN

In many cases the presence of circulating microorganisms in the blood is either a part of the natural history of the infectious disease or a reflection of serious, uncontrolled infection. Depending on the class of agent involved, this process is described as viremia, bacteremia, fungemia, or parasitemia. The terms **sepsis** and **septicemia** refer to the major clinical symptom complexes generally associated with bacteremia. The clinical findings may develop acutely, as in septic shock, or slowly, as in most forms of infective endocarditis. Viremia is usually a very early, even prodromal, event accompanied by fever, malaise, and other constitutional symptoms, such as muscle aches. With the exception of a few specific infections, the detection of viremia does not play a role in the diagnosis or management of viral infections. The presence of bacteremia defines some of the most serious and life-threatening situations in medical practice, and it has a marked impact on the management and outcome of bacterial infections. This chapter will focus on the causes and implications of bacteremia and, to a lesser extent, fungemia. Diseases in which parasitemia is a feature are covered in Chapters 51 to 54.

Sepsis refers to clinical findings

Onset can be insidious or dramatic

Bacteremia or fungemia may also result from microbial growth on the inner or outer surfaces of intravenous devices. Clinical manifestations may be minor initially, but may later become severe. Because the bloodstream is sterile in healthy individuals, bacteremia is considered potentially serious regardless of the symptoms present; however, transient bacteremia may occur when there is manipulation or trauma to a body site that has a normal flora. After such events, species indigenous to the site may appear briefly in the blood, but they are soon cleared. Such transient bacteremias usually have no immediate clinical significance, but they are important in the pathogenesis of infective endocarditis.

Bloodstream is sterile in health

Transient, benign bacteremia is common

## INTRAVASCULAR INFECTION

Intracardiac infections (endocarditis) and those primarily involving veins (thrombophlebitis) or arteries (endarteritis) are usually caused by bacteria, although other agents including fungi and viruses have been occasionally implicated. This discussion will focus primarily on the bacterial causes, because they are the most frequent. Infections of the cardiovascular system are usually extremely serious and, if not promptly and adequately treated, can be fatal. They commonly produce a constant shedding of organisms into the

Primarily caused by bacteria

bloodstream that is often characterized by continuous, low-grade bacteremia (1 to 20 organisms/mL of blood) in untreated patients.

## Infective Endocarditis

Sites of endocardial infection include prosthetic valves

The term **infective endocarditis** is preferable to the commonly used term **bacterial endocarditis,** simply because not all infections of the endocardial surface of the heart are caused by bacteria. Most infections occur on natural or prosthetic cardiac valves, but can also develop on septal defects, shunts (eg, patent ductus arteriosus), or the mural endocardium. Infections involving coarctation of the aorta are also classified as infective endocarditis because the clinical manifestations and complications are similar.

### *Pathogenesis*

The pathogenesis of infective endocarditis involves several factors that, if concurrent, result in infection:

Hemodynamic effects of cardiac abnormalities create sites for attachment

1. The endothelium is altered to facilitate colonization by bacteria and deposition of platelets and fibrin. Most infections involve the mitral or aortic valves, which are particularly vulnerable when abnormalities such as valvular insufficiency, stenosis, intracardiac shunts (eg, ventricular septal defect), or direct trauma (eg, catheters) exist. The turbulence of intracardiac blood flow that results from such abnormalities can lead to further irregularities of the endothelial surfaces that facilitate platelet and fibrin deposition. These factors produce a potential nidus for colonization and infection.
2. Transient bacteremia is common, but it is usually of no clinical importance. Often seen for a few minutes after a variety of dental procedures, it has also been shown to develop after normal childbirth and manipulations such as bronchoscopy, sigmoidoscopy, cystoscopy, and some surgical procedures. Even simple activities such as tooth brushing or chewing candy can cause such bacteremia. The organisms responsible for transient bacteremia are the common surface flora of the manipulated site such as viridans streptococci (oropharynx) and are usually of low virulence. Other, more virulent strains may also be involved, however; for example, intravenous drug abuse may lead to transient bacteremia with *Staphylococcus aureus* or a variety of Gram-negative aerobic and anaerobic bacteria. Whether or not the organisms causing bacteremia (or fungemia) are of high virulence, they can colonize and multiply in the heart if local endothelial changes are suitable.

Transient bacteremia with normal flora is the usual organism source

3. Circulating organisms adhere to the damaged surface, followed by complement activation, inflammation, fibrin, and platelet deposition and further endothelial damage at the site of colonization. The resulting entrapment of organisms in the thrombotic "mesh" of platelets, fibrin, and inflammatory cells leads to a mature vegetation, which protects the organisms from host humoral and phagocytic immune defenses, and to some extent from antimicrobial agents. As a result, the infection can be exceedingly difficult to treat. The vegetation can also create greater hemodynamic alterations in terms of obstruction to flow and increased turbulence. Parts of vegetations may break off and be deposited in smaller blood vessels (embolization) with resultant obstruction and secondary sites of infection. Emboli may be transported to the brain or coronary arteries, for example, with disastrous results.

Bacteria adhere and start development of vegetation

Embolization created by dislodged parts of vegetation

Circulating immune complexes cause peripheral manifestations

Another phenomenon shown to contribute to the infective endocarditis syndrome is the development of circulating immune complexes of microbial antigen and antibody. These complexes can activate complement and contribute to many of the peripheral manifestations of the disease, including nephritis, arthritis, and cutaneous vascular lesions.

Rheumatoid factor and antinuclear antibodies contribute to pathogenesis

Frequently, there is a widespread stimulus to host cellular and humoral immunity, particularly if the infection continues for more than about 2 weeks. This condition is characterized by hyperglobulinemia, splenomegaly, and the occasional appearance of macrophages in the peripheral blood. Some patients develop circulating rheumatoid factor (IgM anti-IgG antibody), which may play a deleterious role by blocking IgG opsonic activity and causing microvascular damage. Antinuclear antibodies, which also appear occasionally, may contribute to the pathogenesis of the fever, arthralgia, and myalgia that is often seen.

In summary, infective endocarditis involves an initial complex of endothelial damage or abnormality, which facilitates colonization by organisms that may be circulating through the heart. This colonization, in turn, leads to the propagation of a vegetation, with its attendant local and systemic inflammatory, embolic, and immunologic complications.

## *Clinical Features*

Infective endocarditis has often been classified by the progression of the untreated disease. **Acute endocarditis** is generally fulminant with high fever and toxicity, and death may occur in a few days or weeks. **Subacute endocarditis** progresses to death over weeks to months with low-grade fever, night sweats, weight loss, and vague constitutional complaints. The clinical course is substantially related to the virulence of the infecting organism; *S. aureus,* for example, usually produces acute disease, whereas infections by the otherwise avirulent viridans streptococci are more likely to be subacute. Before the advent of antimicrobial therapy, death was considered inevitable in all cases. Physical findings often include a new or changing heart murmur, splenomegaly, various skin lesions (petechiae, splinter hemorrhages, Osler's nodes, Janeway's lesions), and retinal lesions.

Acute, subacute, and chronic infective endocarditis determined by virulence of organism

Complications include the risk of congestive heart failure as a result of hemodynamic alterations, rupture of the chordae tendinea of the valves, or perforation of a valve. Abscesses of the myocardium or valve ring can also develop. Other complications relate to the immunologic and embolic phenomena that can occur. The kidney is commonly affected, and hematuria is a typical finding. Renal failure, presumably from immune complex glomerulonephritis, is possible. Left-sided endocarditis can readily lead to coronary artery embolization and "mycotic" aneurysms; the latter will be discussed later in this chapter. In addition, more distant emboli to the central nervous system can lead to cerebral infarction and infection. Right-sided endocarditis often causes embolization and infarction or infection in the lung.

Cardiac, embolic, and immunologically mediated complications lead to death without treatment

## *Etiologic Agents*

Table 68–1 summarizes the most common causes of infective endocarditis. Alpha-hemolytic streptococci and enterococci are involved in just over 50% of the cases. In the so-called culture-negative group, infective endocarditis is diagnosed on clinical grounds, but cultures do not confirm the etiologic agent. This group of patients is difficult to treat, and the overall prognosis is considered poorer than when a specific etiology has been determined. Negative cultures may result from (1) prior antibiotic treatment; (2) fungal endocarditis with entrapment of these relatively large organisms in capillary beds; (3) fastidious, nutritionally deficient, or cell wall–deficient organisms that are difficult to isolate; (4) infection caused by

Streptococci are most common cause

TABLE 68–1

**Common Etiologic Agents in Infective Endocarditis**

| Agent | Approximate Percentage of Cases |
|---|---|
| Viridans streptococci (several species) | 30–40 |
| Enterococci | 5–18 |
| Other streptococci | 15–25 |
| *Staphylococcus aureus* | 15–40 |
| Coagulase-negative staphylococci | 4–30 |
| Gram-negative bacilli | 2–13 |
| Fungi (eg, *Candida, Aspergillus*) | 2–4 |

TABLE 68–2

**Etiologic Agents More Commonly Observed in Special Circumstances**

| Situation | Agent |
| --- | --- |
| Intravenous drug abuse | *Staphylococcus aureus;* enterococci; Enterobacteriaceae and *Pseudomonas;* fungi |
| Prosthetic valve infection | Coagulase-negative streptococci; *S. aureus;* Enterobacteriaceae and *Pseudomonas;* diphtheroids; *Candida* and *Aspergillus* spp. |
| Immunocompromise, chronic illness | Any of the above organisms |

Many explanations for culture-negative endocarditis

obligate intracellular parasites, such as chlamydiae (*Chlamydia psittaci*), rickettsiae (*Coxiella burnetii*), *Rochalimaea* species, or viruses; (5) immunologic factors (eg, antibody acting on circulating organisms); or (6) subacute endocarditis involving the right side of the heart, in which the organisms are filtered out in the pulmonary capillaries.

Some special circumstances alter the relative etiologic possibilities, such as intravenous drug addiction, prosthetic valves, and immunocompromise. The major associations in these cases are summarized in Table 68–2.

## *General Diagnostic Approaches*

Blood culture the most important diagnostic test

The diagnosis of infective endocarditis is usually suspected on clinical grounds; however, the most important diagnostic test for confirmation is the blood culture. In untreated cases, the organisms are generally present continuously in low numbers (1 to 20/mL) in the blood. If an adequate volume of blood is obtained, the first culture will be positive in over 95% of culturally confirmed cases. Most authorities recommend three cultures over 24 hours to ensure detection, and an additional three if the first set is negative. Multiple cultures yielding the same organism support the probability of an intravascular or intracardiac infection. In acute endocarditis, the urgency of early treatment may require collection of only two or three cultures within a few minutes so that antimicrobial therapy can begin.

Echocardiography defines vegetations

Cardiologic procedures such as transthoracic or transesophageal echocardiography can delineate the nature and size of the vegetations and progression of disease. They are also helpful in prediction of some complications such as embolization.

## *General Principles of Management*

Bactericidal antimicrobics required because of protective effect of the vegetation

Antimicrobic combinations often used for synergistic effect

Because of the nature of the lesions and their pathogenesis, response to therapy may be slow and cure is sometimes difficult. Therefore, specific antimicrobial therapy must be aggressive, using agents that are bactericidal (rather than bacteriostatic) and can be given in amounts that achieve high continuous blood levels without causing toxicity to the patient. Treatment may involve a single antimicrobial if the organism is highly susceptible in vitro, or antimicrobial combinations if synergistic effects are possible (eg, a penicillin and an aminoglycoside for enterococcal endocarditis). Parenteral therapy is begun to produce adequate blood levels, and the patient may need to be monitored frequently to ensure antimicrobial activity in the serum sufficient to kill the organisms without causing unnecessary toxicity. Therapy is usually prolonged, lasting longer than 4 weeks in most cases. In some cases, surgery may be required to excise the diseased valve and replace it with a valvular prosthesis. The decision for surgery is sometimes difficult, requiring consultation with both a cardiologist and a surgeon.

Prophylaxis can prevent the development of endocarditis in persons with known congenital or acquired cardiac lesions that predispose to bacterial endocarditis. When they undergo procedures known to cause transient bacteremia (eg, dental manipulations or surgical procedures involving the upper respiratory, gastrointestinal, or genitourinary tracts), administration of high doses of antimicrobics is begun just before the procedure and continued for 6 to 12 hours thereafter. An example of prophylaxis is the case of a patient with rheumatic valvular disease who is planning to undergo dental work. The organism most likely to produce transient bacteremia would be a penicillin-sensitive member of the oral flora, especially viridans streptococci. Thus, an intramuscular dose of penicillin or ampicillin within 30 minutes before the procedure, followed by a high dose of intramuscular penicillin or oral amoxicillin 6 hours later, would be expected to afford protection. Several regimens similar to this approach are recommended, depending on the patient, the nature of the procedure, and the organisms that might be expected to be involved.

Antimicrobial prophylaxis indicated for those with cardiac abnormalities

Antimicrobial prophylaxis used with dental work

## Mycotic Aneurysm

The term **mycotic aneurysm** is somewhat misleading, because it suggests infection by fungi. Originally used by Sir William Osler to describe the mushroom-shaped arterial aneurysm that can develop in patients with infective endocarditis, the term now applies to infection with any organism that causes inflammatory damage and weakening of an arterial wall with subsequent aneurysmal dilatation. This sequence can progress to rupture, with a fatal outcome.

Intra-arterial infection occurs at sites of vascular injury

Arterial infection can result from direct extension of an intracardiac infection or from septic microemboli from a cardiac focus, with seeding of vasa vasorum within the arterial wall. In addition to infective endocarditis, other predisposing factors include damaged arterial intima by atherosclerotic plaques, vascular thrombi, congenital malformations, trauma, or spread from a contiguous focus of infection directly into the artery. The clinical features vary according to the site of involvement. Common findings may include pain at the site of primary arterial supply (eg, back or abdominal pain in abdominal aortic infections) and fever. In many cases, the initial presentation is the result of a catastrophic hemorrhage, particularly intracerebral aneurysms. The etiologic agents, diagnosis, and management are similar to infective endocarditis.

Etiologic agents similar to those of infective endocarditis

## Suppurative Thrombophlebitis

**Suppurative** (or septic) **thrombophlebitis** is an inflammation of a vein wall frequently associated with thrombosis and bacteremia. There are four basic forms: superficial, pelvic, intracranial venous sinus, and portal vein infection (pylephlebitis). With the steadily increasing use of intravenous catheters, the incidence of superficial thrombophlebitis has risen and represents a major complication in hospitalized patients.

Thrombotic site may become seeded with organisms from blood

The pathogenesis involves thrombus formation, which may result from trauma to the vein, extrinsic inflammation, hypercoagulable states, stasis of blood flow, or combinations of these factors. The thrombosed site is then seeded with organisms, and a focus of infection is established. In superficial thrombophlebitis, an intravenous cannula or catheter may cause local venous wall trauma, as well as serve as a foreign body nidus for thrombus formation. Infection develops if bacteria are introduced by intravenous fluid, local wound contamination, or bacteremic seeding from a remote infected site.

Intravenous catheter often associated with thrombophlebitis

Thrombophlebitis of pelvic, portal, or intracranial venous systems most often occurs as a result of direct extension of an infectious process from adjacent structures, or from venous and lymphatic pathways near sites of infection. For example, infections of intracranial venous sinuses usually result from orbital or sinus infections (causing cavernous sinus thrombophlebitis) or from infections of the mastoid and middle ear (causing lateral and sagittal sinus thrombophlebitis). Pelvic thrombophlebitis is a potential result of intrauterine infection (endometritis), particularly after pelvic surgery or 2 to 3 weeks after childbirth. Pelvic or intra-abdominal infections may also spread to the portal venous system to produce pylephlebitis.

Local infection may extend to veins

### *Clinical Features*

Common features often include fever and inflammation over the infected vein. Pelvic or portal vein thrombophlebitis is usually associated with high fever, chills, nausea, vomiting,

TABLE 68–3

**Common Etiologic Agents in Suppurative Thrombophlebitis**

| SITE | AGENT |
|---|---|
| Superficial veins (eg, saphenous, femoral, antecubital) | *Staphylococcus aureus;* Gram-negative aerobic bacilli |
| Pelvic veins, portal veins | *Bacteroides* spp.; microaerophilic or anaerobic streptococci; *Escherichia coli;* beta-hemolytic streptococci (group A or B) |
| Intracranial venous sinuses (cavernous, sagittal, lateral) | *Haemophilus influenzae, Streptococcus pneumoniae;* beta-hemolytic streptococcus (group A); anaerobic or microaerophilic streptococci; S. aureus |

Signs and symptoms depend on anatomic site involved

and abdominal pain. Jaundice may develop in portal vein infections. Intracranial thrombophlebitis varies in its presentation. Headache, facial or orbital edema, and neurologic deficits are variably present; for example, cavernous sinus thrombophlebitis often causes palsies of the third through sixth cranial nerves. Complications include extension of suppurative infection into adjacent structures, further propagation of thrombi, bacteremia, and septic embolization. Embolization from pelvic or leg veins is to the lungs and pulmonary embolism with infarction may be the presenting manifestation of the remote infection.

## *Etiologic Agents*

The major infectious causes of suppurative thrombophlebitis are outlined in Table 68–3. In superficial thrombophlebitis, which often follows intravenous therapy, organisms that are common nosocomial offenders predominate (*S. aureus,* Gram-negative aerobes). Deeper infections are more frequently caused by organisms that reside on adjacent mucous membranes (eg, *Bacteroides* species in intestinal and vaginal sites) or commonly infect adjacent sites (eg, *Haemophilus influenzae* and *S. pneumoniae* in acute otitis media and sinusitis).

## *General Diagnostic Approaches*

Direct culture or blood culture is usually positive

The diagnosis is often suspected on clinical grounds and from associated events known to create predisposition to such infections (eg, surgery, presence of indwelling venous cannulas). Direct cultures of the infected site or blood cultures usually yield the infecting organism, because bacteremia is often present. Radiologic procedures, including scanning methods, may be necessary to localize the process and support the diagnosis. In some cases, surgical exploration is required, both for definitive treatment and to obtain specimens for cultures.

## *General Principles of Management*

Antimicrobic therapy and removal of catheters

The choice of antimicrobial agents is based on culture and susceptibility test results, or in the absence of microbiologic data, the most likely possibilities listed in Table 68–3. Other important aspects of management include prompt removal of possible offending sources, such as intravenous catheters, vigorous treatment of adjacent infections, and sometimes surgical excision and drainage. Severe cases may also benefit from systemic anticoagulant therapy to prevent further propagation of thrombi and embolization.

Many cases are preventable. Unnecessary, long-term intravenous cannulation should be avoided. Whenever possible, it is better to use short needles such as "scalp vein" cannulas than venous catheters or plastic cannulas. Careful asepsis is essential with all

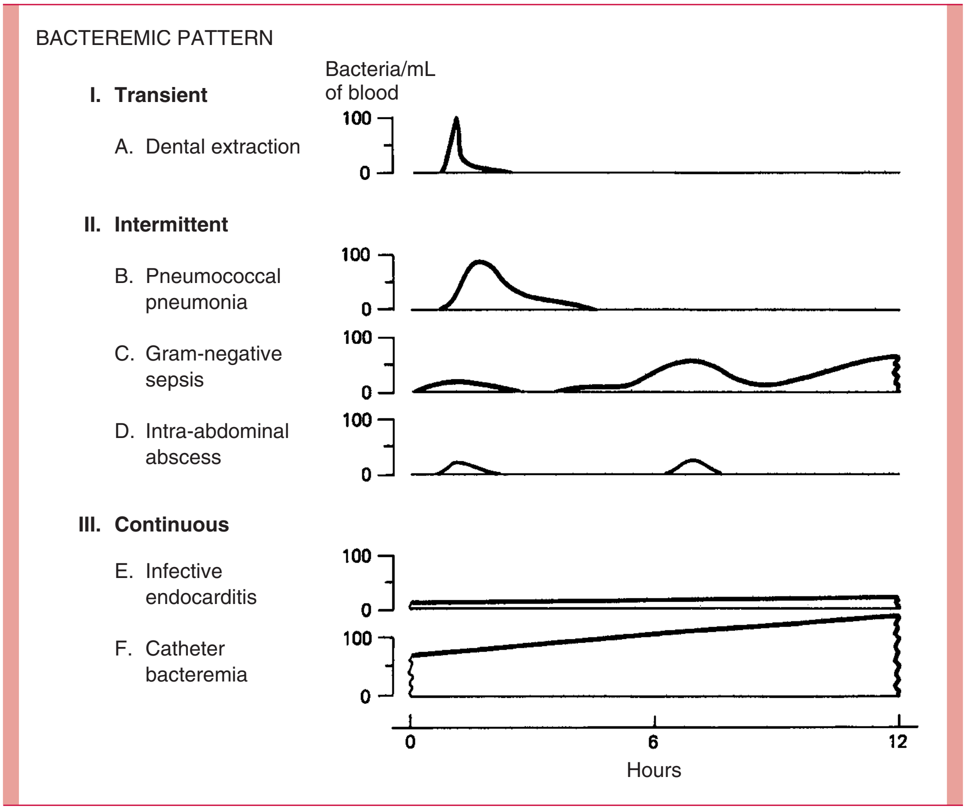


FIGURE 68–1

Patterns of bacteremia. The magnitude and timing of bacteremia for six typical patients (A–F) are depicted. These findings have implications for blood culture sampling plans. Cases such as A and B are detected only by cultures taken early in their course. Cases such as C and particularly D are more variable and more likely to be detected by cultures spaced over the time period shown. Continuous bacteremia (E and F) should be detected by any sampling plan. It could be confused with transient bacteremia on single blood cultures, because both are caused by organisms of low virulence (viridans streptococci, *Staphylococcus epidermidis*); in cases such as E and F, however, bacteremia is sustained, whereas cases of transient bacteremia yield multiple positive results only if they are collected at or near the same time.

intravenous procedures to prevent contamination of intravenous fluids, tubing, and the site of venous entry.

## Intravenous Catheter Bacteremia

A variant of intravascular infection develops when a medical device such as an intravenous catheter or any of several types of monitoring devices placed in the bloodstream becomes colonized with microorganisms. The event itself does not have immediate clinical significance but, unlike transient bacteremia from manipulation of normal floral sites, the bacteremia continues. This persistence greatly increases the chances of secondary complications such as infective endocarditis and metastatic infection, depending on any underlying disease and the virulence of the organism involved.

Significant endocarditis and metastatic infection risk

The organisms involved are usually those found in the skin flora, such as *S. epidermidis, Corynebacterium jeikeium,* or *S. aureus.* In debilitated patients already on antimicrobial therapy, *Candida* species may be involved. Occasionally, the sources of contamination are the intravenous solutions themselves rather than the skin. In these cases, members of the Enterobacteriaceae, *Pseudomonas,* or other Gram-negative rods are more likely.

Skin flora most commonly involved

The clinical findings in catheter bacteremia are usually mild despite large numbers of organisms in the bloodstream (Fig 68–1). In addition to low-grade fever, signs of inflammation may or may not be present. Management includes removal of the contaminated catheter. Antimicrobial therapy alone often does not eradicate the organisms in the presence of a foreign body (the catheter).

Removal of contaminated catheter usually necessary

## BACTEREMIA FROM EXTRAVASCULAR INFECTION

Although bacteremia is an integral feature of intravascular infection, most cases of clinically significant bacteremia are the result of overflow from an extravascular infection. In these cases, the organisms drained by the lymphatics or otherwise escaping from the infected focus reach the capillary and venous circulation through the lymphatic vessels. De-

Bacteremia may be high despite mild manifestations

Bacteremia is more variable than with intravascular infection

pending on the magnitude of the infection and the degree of local control, these organisms may be filtered in the reticuloendothelial system or circulate more widely, producing bacteremia or fungemia. The process is dependent on the timing and interaction of multiple events and is thus much less predictable than intravascular infection. If the infection is extensive and uncontrolled, such as an overwhelming staphylococcal pneumonia, there may be hundreds or even thousands of organisms per milliliter of blood, a poor prognostic sign. An intra-abdominal abscess may only seed a few organisms intermittently until it is discovered and drained. Most infections that produce bacteremia fall between these extremes, with bloodstream invasion more common in the acute phases and intermittent at other times.

Frequently associated with severe infections such as meningitis

The causative organisms and the frequencies with which they usually produce bacteremia (or fungemia) are listed in Table 68–4. There is considerable overlap, and the probability of bacteremia is dependent on the site as well as the organism. Any organism producing meningitis is likely to produce bacteremia at the same time. Infections with *H. influenzae* type b are usually bacteremic, whether the site is the meninges, epiglottis, or periorbital tissues. Meningitis caused by *S. pneumoniae* can be expected to be bacteremic, but only 20 to 30% of patients with pneumococcal pneumonia have positive blood cultures.

Bacteremia is overflow from respiratory, urinary, wound, and other primary sites of infection

The most common sources of bacteremia are urinary tract infections, respiratory tract infections, and infections of skin or soft tissues, such as wound infections or cellulitis. The frequency with which any organism causes bacteremia is related to both its propensity to invade the bloodstream (see Table 68–4) and how often it produces infections. For example, cases of *Escherichia coli* bacteremia are common, attributable in part to the fact that *E. coli* is the most frequent cause of urinary tract infection.

TABLE 68–4

**Frequency of Detection of Bloodstream Invasion by Bacteria and Some Fungi during Significant Infections at Extravascular Sites**

| | |
|---|---|
| **LARGE (>90%) PROPORTION OF CASES** | |
| *Haemophilus influenzae* type b | *Brucella*[a] |
| *Neisseria meningitidis* | *Salmonella typhi* |
| *Streptococcus pneumoniae* (meningitis) | *Listeria* |
| **VARIABLE (10–90%) DEPENDING ON STAGE AND SEVERITY OF INFECTION** | |
| Beta-hemolytic streptococci | Enterobacteriaceae |
| *S. pneumoniae* | *Pseudomonas* |
| *Staphylococcus aureus* | *Bacteroides* |
| *Neisseria gonorrhoeae* | *Clostridium* (myositis and endometritis) |
| *Leptospira*[a] | Anaerobic cocci |
| *Borrelia*[a] | *Candida* |
| *Acinetobacter* | *Cryptococcus neoformans*[a] |
| *Shigella dysenteriae* | |
| **SMALL (<10%) PROPORTION OF CASES** | |
| *Shigella* (except *S. dysenteriae*) | *Pasteurella multocida* |
| *Salmonella enteritidis* | *Haemophilus,* nonencapsulated |
| *Campylobacter jejuni*[a] | |
| **ISOLATION TOO RARE TO JUSTIFY ATTEMPT** | |
| *Vibrio* (intestinal infections) | *Clostridium tetani* |
| *Corynebacterium diphtheriae* | *Clostridium botulinum* |
| *Bordetella pertussis* | *Clostridium difficile* |
| *Mycobacterium*[b] | *Legionella*[c] |

[a] Isolation and/or demonstration requires special methods or prolonged incubation.

[b] *Mycobacterium avium-intracellulare* infections in AIDS patients often yield positive results.

[c] Infrequent isolation may be due to inadequate cultural methods.

## SEPSIS AND SEPTIC SHOCK

Bacteremia is the presence of viable bacteria circulating in the blood. When signs and symptoms result, further terms are used to delineate the progression of potential consequences that may occur. Both Gram-negative and Gram-positive organisms can produce the same findings, as well as fungi, protozoa, and even some viruses.

Associated with bacteremic Gram-negative and Gram-positive infections

**Sepsis** is the suspicion (or proof) of infection and evidence of a systemic response to it (eg, tachycardia, tachypnea, hyperthermia, or hypothermia). The **sepsis syndrome** includes findings of sepsis plus evidence of altered organ perfusion. These can include reduction in urine output, mental status changes, systemic acidosis, and hypoxemia. If the process remains uncontrolled, there is subsequent progression to **septic shock** (development of hypotension); **refractory septic shock** (hypotension not responsive to standard fluid and pharmacologic treatment); and **multiorgan failure,** including major target organs such as the kidneys, lungs, and liver, and disseminated intravascular coagulation. Mortality is exceedingly high when patients develop refractory septic shock or multiorgan failure.

Sepsis syndrome progresses through shock to organ failure

The initial events in the sepsis syndrome appear to be vasodilatation with resultant decreased peripheral resistance and increased cardiac output. The patient is flushed and febrile. Capillary leakage and reduced blood volume follow, leading to a whole series of events identical to those seen in shock resulting from blood loss. These manifestations include vasoconstriction, reflex capillary dilatation, and local anoxic damage. Once this stage is reached, the patient may develop hypotension and hypothermia, and acidosis, hypoglycemia, and coagulation defects ensue with failure of highly perfused organs such as the lungs, kidneys, heart, brain, and liver.

Vasodilatation is followed by complex response

The mechanisms involved in development of septic shock have been studied extensively in experimental animals. Most of the features seen in humans can be produced with the lipopolysaccharide endotoxin of the Gram-negative cell wall, although there is some variation between animal species and with different preparations. The various events that occur are complex. They include (1) release of vasoactive substances such as histamine, serotonin, noradrenaline, and plasma kinins, which may cause arterial hypotension directly and facilitate coagulation abnormalities; (2) disturbances in temperature regulation, which may be due to direct central nervous system effects or, in the case of the early febrile response, mediated by interleukin 1 (IL-1) and tumor necrosis factor (TNF) released from macrophages; (3) complement activation and release of other inflammatory cytokines by macrophages (eg, IL-2, IL-6, IL-8, and interferon-gamma); (4) direct effects on vascular endothelial cell function and integrity; (5) depression of cardiac muscle contractility by TNF, myocardial depressant factor, and other less well-defined serum factors; and (6) impairment of the protein C anticoagulation pathway, resulting in disseminated intravascular coagulation. The resultant alterations in blood flow and capillary permeability lead to progressive organ dysfunction.

Endotoxin causes release of vasoactive substances

Cytokines, complement, and other mediators have physiologic effects

Early recognition of the problem is critical, and management obviously requires considerably more than antimicrobial therapy. Other primary therapeutic measures include maintenance of adequate tissue perfusion through careful fluid and electrolyte management and the use of vasoactive amines. There is also evidence that protein C replacement may ameliorate the coagulopathy.

Antimicrobic, fluid, and coagulation management are crucial

## BLOOD CULTURE

The primary means for establishing a diagnosis of sepsis is by blood culture. The microbiologic principles involved are the same as with any culture. A sample of the patient's blood is obtained by aseptic venipuncture and cultured in an enriched broth or, after special processing, on plates. Growth is detected, and the organisms are isolated, identified, and tested for antimicrobial susceptibility. Because of the importance of blood cultures in the diagnosis and therapy of most bacterial and fungal infections, considerable attention must be paid to details of sampling if the prospects of obtaining a positive culture are to be maximized. The approach to blood culture must be tailored to the individual patient; no single procedure is best for all individuals. The important features are described below.

Importance of blood culture demands attention to details

## Blood Culture Sampling

### *Venipuncture*

Skin decontamination removes bulk of skin flora

Some anticoagulants have antimicrobial properties

Before venipuncture, the skin over the vein must be carefully disinfected to reduce the probability of contamination of the blood sample with skin bacteria. Although it is not possible to "sterilize" the skin, quantitative counts can be markedly reduced with a combination of 70% alcohol and an iodine-based antiseptic. Mechanical cleansing is as important as use of the antiseptic. Poor phlebotomy technique such as repalpating the vein after the preparation is related to introduction of contaminants. Blood is ideally drawn directly into a blood culture bottle or a sterile blood collection vacuum tube containing an anticoagulant free of antimicrobial properties. Sodium polyanethol sulfonate is currently preferred; other anticoagulants such as citrate and ethylenediaminetetraacetic acid have antibacterial activity. Blood should not be drawn through indwelling venous or arterial catheters unless it cannot be obtained by venipuncture.

### *Volume*

Number of organisms in blood often <1 organism/mL

The number of organisms present in blood is often low (<1 organism/mL) and cannot be predicted in advance. Thus, small samples yield fewer positive cultures than larger ones. For example, as the volume sampled increases from 2 to 20 mL, the diagnostic yield increases by 30 to 50%. Samples of at least 10 mL should be collected from adult patients. The same principles apply with infants and young children, but the sample size must be reduced to take account of the smaller total blood volume of a child. Although it should be possible to obtain at least 1 mL, smaller volumes should still be cultured because bacteremia at levels of more than 1000 bacteria/mL is found in some infants.

### *Number*

Two or three blood cultures usually adequate

If the volume is adequate, it is rarely necessary to collect more than two or three blood cultures to achieve a positive result. In intravascular infections (eg, infective endocarditis), a single blood culture is positive in more than 95% of cases. Studies of sequential blood cultures from bacteremic patients without endocarditis have yielded 80 to 90% positive results on the first culture, more than 90 to 95% with two cultures, and 99% in at least one of a series of three cultures.

### *Timing*

Timing of intermittent bacteremia not predictable

Antimicrobic therapy may interfere with blood culture results

The best timing schedule for a series of two or three blood cultures is dependent on the bacteremic pattern of the underlying infection and the clinical urgency of initiating antimicrobial therapy. Figure 68–1 illustrates some typical bacteremic patterns that can be related to the probability of obtaining positive blood cultures. Transient bacteremia is usually not detected, because organisms are cleared before the appearance of any clinical findings suggesting sepsis. The continuous bacteremia of infective endocarditis is usually readily detected, and timing is not critical. Intermittent bacteremia presents the greatest challenge because fever spikes generally occur after, rather than during, the bacteremia. Little is known about the periodicity of bloodstream invasion, except that the bacteremia is more likely to be present and sustained in the early acute stages of infection. Closely spaced samples are less likely to detect the organism than those spaced an hour or more apart. In urgent situations, when antimicrobial therapy must be initiated, two or three samples should be collected at brief intervals and therapy begun as soon as possible. It is generally not useful to collect blood cultures while the patient is receiving antimicrobics unless none were collected before therapy or there is a change in the clinical course suggesting superinfection. The laboratory should be advised when such cultures are submitted, because it is sometimes possible to inactivate an antimicrobic, for example, with beta-lactamases.

## Laboratory Processing

The basic blood culture procedure of incubating blood in an enriched broth is quite simple, but considerable effort must be expended to ensure detection of the broadest range of organisms in the least possible time. Daily examination of cultures for 1 week or more and a routine schedule of stains and/or subcultures of apparently negative cultures are required to detect organisms such as *H. influenzae* or *N. meningitidis,* which usually do not produce visual changes in the broth. Direct plating of blood onto blood or chocolate agar is accomplished in a system that concentrates the blood by centrifugation following lysis of the erythrocytes. This is particularly useful for bacterial quantification and rapid identification. Automated blood culture systems detect metabolic activity (primarily $CO_2$ generation) in broth culture for initial detection in place of the conventional visual and staining examinations. These systems detect growth sooner than conventional methods but still require subculture for confirmation, identification, and susceptibility testing.

Blood added to enriched broth

Automated and direct plating procedures now available

Isolation of fungi is favored by ensuring maximum aerobic conditions in direct plating systems and broth bottles. Conversely, anaerobes are recovered best when a highly reduced environment is provided for plates and broths. Some bacteria, such as *Leptospira,* are not isolated by routine blood culture procedures. The laboratory must be notified in advance so special media can be used.

Special cultural conditions required for yeasts and anaerobes

Because the blood is normally sterile, the interpretation of blood cultures growing a pathogenic organism is seldom a problem. The major problem is the differentiation of agents causing transient bacteremia and skin contamination from those opportunists associated with an intravascular or extravascular infection. Transient bacteremia is of short duration (see Fig 68–1), is associated with manipulation of or trauma to a site possessing a normal flora, and involves species indigenous to that site. Despite skin disinfection, 2 to 4% of venipunctures result in contamination of the culture with small numbers of cutaneous flora such as *S. epidermidis,* corynebacteria (diphtheroids), and propionibacteria. The presence of these organisms in blood cultures can be considered a result of skin contamination unless quantitative procedures indicate large numbers (>5 organisms/mL) or repeated cultures are positive for the same organism. These findings should suggest diseases such as infective endocarditis or catheter bacteremia.

Interpretation involves distinguishing infection from normal skin flora contamination

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CHAPTER 69

# Infections of the Fetus and Newborn

C. George Ray

The usual 10-month period from conception through birth and the first 4 weeks of extrauterine life is one of unusual susceptibility to infection but also a time at which special defenses acquired from the mother are operating.

1. During normal development, the fetus is in a protected intrauterine environment, with fetal membranes serving as a physical barrier to external infection and the placenta contributing, with maternal immunity, to protection against many blood-borne infections. Transplacental transmission of specific immunoglobulins, particularly of the IgG class (IgM does not normally cross the placental barrier), continues to provide some immunologic protection to the infant for weeks to months after birth, while cytokines from the mother can provide transient cell-mediated immune support. If the infant is breast-fed, specific immunoglobulins (predominantly of the IgA class) in maternal colostrum afford some protection against pathogens that involve or invade through the infant's gastrointestinal tract.

Fetus protected in intrauterine environment

Passive immunity is acquired from mother

2. On the other hand, the fetal immune system is immature, and there is relative suppression of maternal cell-mediated immunity as pregnancy progresses. These immune deficiencies serve an important biological purpose; they protect fetus and mother from activation of specific immunologic recognition and response mechanisms to differences in their histocompatibility locus antigens. If these processes did not occur normally, the fetus could be immunologically rejected by the mother or the fetal immune mechanisms activated to respond against maternal antigens in a form of "graft versus host" disease.

Fetal immune system immature and maternal cell-mediated immunity suppressed

3. Specific and nonspecific immune responses begin to develop in early fetal life, perhaps as early as 8 weeks' gestation; however, a nearly normal immunocompetent state is usually not achieved until the infant is more than 2 years of age. Deficiencies commonly seen in the early period include poor antibody response to polysaccharide antigens, decreased phagocytic capability and variability in intracellular killing of certain infectious agents, lower levels of complement components, and decreased opsonic capacity.

Specific deficiencies of neonate include poor T cell–independent responses

4. Cell growth and organ differentiation are at their highest rates in the fetal–neonatal period, making the host especially susceptible to permanent damage when an infectious process intervenes.

Infection may have teratogenic effects

The actual risk of infection and the types of pathogens encountered are influenced by a variety of interacting factors, including the state of maternal health and susceptibility to specific agents, adequacy of fetal and neonatal nutrition, integrity of fetal membranes, and degree of maturity at birth. This chapter outlines the major types of infection of concern to

Risk of infection influenced by fetal and maternal factors those caring for the fetus and neonate and the general approaches to their diagnosis. Specific biological characteristics and aspects of prevention and treatment for each of the agents have been addressed in previous chapters.

## DEFINITIONS

Infection can occur prenatally, natally, or postnatally

A number of terms are commonly used to describe the infections that can affect the fetus and newborn. **Prenatal** infections include those acquired by the mother and/or fetus at any time before birth. When fetal infection develops, it is usually blood-borne to the placenta with subsequent spread to the fetus (transplacental) or follows the ascending route from the vagina through torn or ruptured fetal membranes. **Natal,** or peripartum, infections are those acquired during delivery. They are often caused by agents in the maternal genital tract but occasionally result from organisms introduced from exogenous sources through attendants, fetal monitors, or other instruments. **Postnatal** infections, which constitute the remainder of the group, include all infections acquired after delivery throughout the newborn (or neonatal) period, defined as the first 4 weeks of life.

Congenital infections acquired prenatally or during delivery

Another commonly used term is **congenital** infection, which describes infection occurring at any time before or at birth (prenatal or natal). Consequently, the infection is usually still active in the newborn period and sometimes persists for months or years. **Perinatal** infection is often used to include a period extending from 20 to 28 weeks' gestation to 7 to 28 days after birth. The term will not be used in this chapter.

Prolonged rupture of membranes enhances risk of chorioamnionitis

**Chorioamnionitis** is an inflammatory response to infectious agents involving the chorionic and amniotic fetal membranes. It usually results from entry of pathogens from the vagina through tears or ruptures in the membranes, and it places the fetus at risk of direct exposure just before or at delivery. The risk of chorioamnionitis increases rapidly when membranes have been ruptured for longer than 12 hours before birth. When infection is by the blood-borne maternal route, there may be evidence of infection of the placenta, termed **placentitis. Endometritis** may be observed occasionally if the infection is an extension from a maternal pelvic focus along venous or lymphatic pathways. **Sepsis** is a term used to indicate a severe systemic bacterial infection associated with bacteremia.

## COMMON ETIOLOGIC AGENTS

Rarity of some childhood infections in infancy related to exposure and passive immunity

*S. aureus* infections are typically postnatal

Table 69–1 lists the major pathogens affecting the fetus and newborn, according to the usual modes of acquisition. Some, such as *Mycobacterium tuberculosis* and *Plasmodium* species, are exceedingly rare, but require consideration in certain clinical and epidemiologic circumstances. It should also be noted that some pathogens that commonly affect older infants and children are quite rarely observed in newborns. This phenomenon is partially attributable to the protective effect of maternally derived immunity to organisms such as *Haemophilus influenzae* type b, *Streptococcus pneumoniae, Neisseria meningitidis,* and mumps and measles viruses but also reflects less opportunity for exposure to some agents early in life. Some organisms, such as *Staphylococcus aureus,* rarely cause prenatal or natal infections but commonly colonize in the postnatal period and most often cause disease after the first week of life.

First exposure is to pathogens in maternal genital flora

If one views the fetus as existing normally in a protected, "germ-free" intrauterine environment before emerging into a milieu of potential pathogens, it is easy to see how the newborn can be colonized with the first organisms encountered, some of which can cause disease. The external pathogenic flora initially acquired can include organisms frequently present in the maternal genital tract, such as group B streptococci and *Escherichia coli,* as well as less common *Neisseria gonorrhoeae, Listeria monocytogenes, Chlamydia trachomatis,* and herpes simplex virus, all of which are important causes of natal infection.

Human and environmental factors determine common neonatal infections

Postnatal infections may be late manifestations resulting from prenatal or natal colonization by pathogens such as those mentioned previously, but additional organisms may be acquired after birth. Particular risks include contamination of the nursery environment by a variety of Gram-negative bacteria, staphylococci, and some common viruses (see Table 69–1) and attendants who are infected with or carrying such organisms. The risks are increased if the infant is born prematurely or otherwise physically compromised, and

TABLE 69–1

**Modes of Infection and Major Agents**

| | Agents | | |
|---|---|---|---|
| Mode | Bacteria | Viruses | Other |
| Prenatal transplacental | *Listeria monocytogenes, Mycobacterium tuberculosis* (rare), *Treponema pallidum* | Rubella, cytomegalovirus, enteroviruses, Epstein–Barr virus, human immunodeficiency virus, parvovirus B19, lymphocytic choriomeningitis virus | *Toxoplasma gondii, Plasmodium* spp. |
| Ascending | Group B streptococci, *Escherichia coli, L. monocytogenes* | Cytomegalovirus, herpes simplex | *Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum* |
| Natal | Group B streptococci; *E. coli, L. monocytogenes, Neisseria gonorrhoeae* | Herpes simplex, cytomegalovirus, enteroviruses, hepatitis B, varicella–zoster, human immunodeficiency virus | *C. trachomatis* |
| Postnatal | E. coli, group B streptococci, *L. monocytogenes,* miscellaneous Gram-negative bacteria, *Staphylococcus aureus, Staphylococcus epidermidis, Clostridium tetani* | Cytomegalovirus, herpes simplex, enteroviruses, varicella–zoster, respiratory syncytial virus, influenza viruses, human immunodeficiency virus | |

they are amplified by prolonged hospitalization and invasive procedures such as respiratory intubation, mechanical ventilation, and intravenous treatment, as well as by blood or blood product transfusions.

Prematurity, prolonged hospitalization, and invasive procedures increase risk

## EFFECT OF PRENATAL INFECTION ON PREGNANCY AND INTRAUTERINE DEVELOPMENT

All of the agents indicated in Table 69–1 as causing prenatal infections have the potential of creating an adverse pregnancy outcome, either as a result of compromising the health of the mother or by directly affecting the fetus. The effect can be untimely termination of pregnancy resulting in abortion, stillbirth, or prematurity, as well as developmental defects and fetal malnutrition.

## CLINICAL FEATURES, DIAGNOSIS, AND MANAGEMENT

### Acute Bacterial Sepsis

When a physician first encounters a sick newborn, the primary concern is whether the illness represents sepsis and/or meningitis caused by bacteria. This determination is important, because treatment is both feasible and extremely urgent. Clinical disease apparent at birth or developing within the first 3 days of life (early onset) has usually been acquired prenatally. Mortality can exceed 70%, even with prompt treatment. Later onset of symptoms is commonly associated with natal or postnatal acquisition of pathogens; however, these infections can also be severe. If meningitis develops, the overall mortality, even with treatment, ranges from 10 to 25%, and permanent neurologic damage may occur in 30 to 50% of survivors. The two pathogens most commonly associated with neonatal sepsis and meningitis are group B streptococci and *E. coli.*

Early-onset neonatal infections may have 70% mortality

Group B streptococcal and *E. coli* sepsis and meningitis are most common

The diagnosis of neonatal infections is based first on clinical suspicion. There is sometimes a history of recent maternal febrile illness immediately before or at birth. Other suggestive features include fetal distress, prolonged rupture of membranes (>12 hours), foul-smelling amniotic fluid, and premature delivery. The first signs and symptoms of illness in the infant may be subtle and extremely variable, including respiratory distress, apneic episodes, cyanosis, irritability, unexplained jaundice, tachycardia, poor feeding, abdominal distention, and fever. Initial laboratory findings often include either leukocytosis, with an increased proportion of immature neutrophils, or leukopenia. The development of seizures, hypotension, or disseminated intravascular coagulation indicates a particularly grave prognosis.

Prematurity and prolonged rupture of membranes are risk factors

Clinical clues subtle in newborn

Diagnostic tests for suspected infections must be initiated as quickly as possible, followed by empirical antimicrobial therapy while waiting for culture results. The major tests include examination and culture of cerebrospinal fluid and blood culture. The antimicrobics initially chosen are those known to be effective against the pathogens most commonly encountered. They often include ampicillin for the streptococci (also useful for *L. monocytogenes*) and an aminoglycoside such as gentamicin for *E. coli.*

Blood and cerebrospinal fluid culture performed initially

## Other Bacterial and Chlamydial Infections

Although *N. gonorrhoeae* and *C. trachomatis* are common natally acquired infections, they are usually not associated with sepsis. Both can produce a severe conjunctivitis in the newborn that requires prompt diagnosis and treatment. Gonococcal ophthalmia is usually apparent in the first 5 days after birth, whereas the onset of chlamydial conjunctivitis is frequently delayed until after the first week of life. Another significant illness associated with natally acquired *C. trachomatis* infection is infant pneumonia syndrome. The onset of respiratory symptoms is often delayed, with most cases occurring between 2 weeks and 6 months of age. This illness is also considered in Chapter 30.

*C. trachomatis* and gonococci produce severe conjunctivitis

Chlamydia infant pneumonia syndrome occurs in infants up to 6 months of age

Localized infections, such as cutaneous or subcutaneous abscesses, show a particular association with postnatally acquired *S. aureus* and occasionally with various Gram-negative bacteria. If the newborn is affected by a staphylococcal strain that produces exfoliative toxin, the local lesion may be relatively trivial in contrast to the more widespread effect of circulating toxin on the skin, which is termed **staphylococcal scalded skin syndrome.** Prompt treatment with an antistaphylococcal antimicrobial agent results in resolution of the disease within 2 weeks, usually with complete healing.

Postnatal infections by *S. aureus* may cause scalded skin syndrome

## Syphilis

If prenatal infection by *Treponema pallidum* (congenital syphilis) is left untreated, the result can be long-term damage, often without apparent signs or symptoms in the newborn period. To minimize these risks, serologic screening is recommended for all pregnant women when first seen in early gestation and at delivery. In addition, serologic testing is recommended whenever clinical or epidemiologic circumstances suggest the possibility of exposure at any time during pregnancy. Prompt treatment of infected mothers during pregnancy, preferably with penicillin, markedly reduces the risk of fetal infection. Similar treatment is also effective for the infected infant.

Risk of congenital syphilis reduced by serologic screening and treatment during pregnancy

# TORCH COMPLEX

When bacterial, spirochetal, and chlamydial infections have been reasonably excluded from consideration, other possibilities can best be remembered by the convenient acronym **TORCH** (**t**oxoplasmosis, **o**ther [viruses], **r**ubella, **c**ytomegalovirus, **h**erpes simplex). This term comprises major infections that can be particularly severe if acquired prenatally. There is often significant overlap of clinical manifestations associated with the various agents in the TORCH complex. Common features may include low birth weight, rash, jaundice, and hepatosplenomegaly. On the other hand, many newborn infants with TORCH infections can go undiagnosed, because the clinical signs may not appear until weeks, months, or even years later. For example, congenital cytomegalovirus infection may be manifested only as mild mental retardation and/or hearing loss that may not

Toxoplasma, rubella, cytomegalovirus, and herpes simplex are all common congenital pathogens become apparent until after the first year of life. Toxoplasmosis also presents a dilemma. It is estimated that as many as 1 in 200 pregnancies in the United States is complicated by primary infection with *Toxoplasma gondii,* which is usually subclinical. Of these cases, approximately 30 to 40% result in fetal infection, but only 8 to 11% of the infected offspring demonstrate clinical symptoms in the newborn period. The remainder are at risk, however, and can ultimately develop neurologic deterioration and/or chorioretinitis, which may not be recognized until 5 or more years later. These observations only partially illustrate the importance of TORCH complex infections and our relative impotence in controlling many of them.

Clinical manifestations may be delayed for years

Of the array of miscellaneous agents grouped in the "other" category, three viruses deserve specific mention. If the mother has active infection with hepatitis B virus during pregnancy, the risk of natal or postnatal transmission to the infant is high (range, 20 to 80%, depending on the status of virus activity). Although it is unlikely that clinical disease will be apparent in the newborn period, it is important to promptly undertake specific measures to prevent infection in the infant when the mother is infected. They include administration of hepatitis B immune globulin immediately after birth as well as immunization of the infant with hepatitis B vaccine. The chance of maternal transmission of the human immunodeficiency virus (HIV), either transplacentally or natally, is estimated to be between 13 and 40%. Prenatal antiretroviral treatment of infected mothers can reduce this risk by 60 to 70%. Primary varicella is infrequent in pregnancy. If the mother develops varicella less than 5 days before or 2 days after delivery, however, the risk of severe neonatal varicella is significant, with a mortality of approximately 20%. It is recommended that the infant be given varicella–zoster immune globulin (or zoster immune globulin) immediately in an attempt to prevent or modify subsequent disease. Maternal zoster infections are not associated with a significant risk to the offspring, presumably because of adequate transplacental transmission of specific antibody.

Hepatitis B infection prevented by immune globulin and vaccine administration

HIV transmission reduced by prenatal antiviral treatment

Neonatal varicella from infected mother is severe if contracted perinatally

The approach to a suspected TORCH complex infection requires some thought in selection of appropriate tests. Table 69–2 summarizes the major clinical and historic features of specific agents and the diagnostic procedures that can be used. The following general comments should also be kept in mind:

1. Clinical and epidemiologic data are used as much as possible in ascertaining likely specific agents.
2. Probabilities must be weighed; for example, congenital cytomegalovirus infection is by far the most frequent TORCH complex agent encountered in the United States (>90% of all proved cases).
3. Potentially treatable infections must be considered first. If toxoplasmosis or herpes simplex is suggested by the historic and clinical findings, it may be controlled by prompt and aggressive therapy. Early identification and treatment of HIV-1 infections can significantly improve long-term prognosis in infants. Other infections, which are potentially preventable by early specific immunoglobulin therapy of the infant, include maternal varicella and hepatitis B infections. The remaining agents involved in the TORCH array are not amenable to specific therapy at present. Their importance lies more in long-term prognosis, planning of continuing care, and epidemiologic management.
4. Serologic testing, when indicated, should be performed on both infant and maternal sera collected at the same time to facilitate interpretation of specific antibody titer levels in the infant. This approach is based on the following principles: passive transplacental transmission of IgG antibodies occurs, but these maternal antibodies normally wane and disappear in the infant over 3 to 6 months. If the infant is actively infected, it usually produces its own specific antibodies to the agent, which then persist for much longer periods. Thus, a specific antibody titer in the infant's serum during the first month of life equal to or less than that of the mother may merely reflect passive transfer and does not support a diagnosis of active infection. On the other hand, if the infant's titer is significantly higher than the mother's (fourfold or greater) or rises progressively in serial samples obtained in later months, active infection by the agent in question is suggested.

Cytomegalovirus is most common congenital infection

Focus on treatable conditions

Comparison of infant and maternal antibody titers aids diagnosis

TABLE 69–2

### TORCH Complex: Salient Features and Diagnostic Tests

**Toxoplasmosis**

**Suggestive clinical findings:** chorioretinitis (found in more than 90% of symptomatic neonatal cases); lymphadenopathy

**Maternal history:** usually negative; occasional cervical lymphadenopathy during pregnancy

**Tests of choice:** culture, PCR, specific maternal and infant antibody titers; follow-up titers may be helpful

**Other Infections**

The list of causes includes enteroviruses, hepatitis B, human immunodeficiency virus, varicella–zoster, Epstein–Barr virus, parvovirus B19, lymphocytic choriomeningitis virus, malaria, and tuberculosis. As the agents in this category most commonly encountered are the enteroviruses, the features summarized here pertain primarily to them.

**Suggestive clinical findings:** sepsis-like syndromes; meningitis; myocarditis (findings are variable)

**Maternal history:** fever common at or near parturition

**Tests of choice:** viral cultures of throat, rectum, and cerebrospinal fluid; rapid PCR analysis of cerebrospinal fluid and other body fluids

**Rubella**

**Suggestive clinical findings:** congenital malformations, often multiple. In severe cases, "celery stalking" of metaphyses of long bones may be seen in early radiographs (see also cytomegalovirus).

**Maternal history:** rubella-like illness or epidemiologic history of exposure in early pregnancy is common. If available, maternal serologic and immunization history can aid in supporting or refuting this diagnostic possibility.

**Tests of choice:** maternal and infant antibody titers, including IgM-specific antibody testing in the infant; serial determinations over 6 months may be of additional help

**Cytomegalovirus**

**Suggestive clinical findings:** none very specific in differentiating infection from most others in the group. Statistically, cytomegalovirus is the most common congenital infection encountered. In florid cases, early radiographs of the long bones may resemble those of congenital rubella (celery stalking).

**Maternal history:** usually none; occasionally, an account of a mononucleosis-like syndrome may be elicited

**Tests of choice:** urine culture (most sensitive test). If results are negative, this diagnosis is highly unlikely; if positive, the diagnosis is supported (especially if cultures are done in the first 3 weeks of life). With advancing age of the infant, however, positive cultures may require careful interpretation before an unequivocal diagnosis is made.

**Herpes Simplex**

**Suggestive clinical findings:** cutaneous vesicles and/or ocular or mucous membrane ulcerations; however, these lesions may not become apparent until other signs of illness have developed

**Maternal history:** up to 70% have no history of genital lesions or symptoms. Others may have a history of recent primary symptomatic infection. It is also important to ascertain whether genital lesions were known to exist in recent sexual partners.

**Tests of choice:** culture of lesions; immunofluorescent and cytologic studies may be available for rapid diagnosis. Throat, urine and cerebrospinal fluid culture, and rapid PCR testing of cerebrospinal fluid and blood are also helpful. Maternal cultures, if positive, may give indirect support regarding etiology.

*Abbreviation:* PCR, polymerase chain reaction.

Infant IgM-specific antibodies suggest active infection

In active congenital and neonatal infections, the infant's early responses often include IgM antibodies. Maternal IgM antibodies rarely cross the placental barrier, so specific IgM antibody determinations early in life may be useful for the diagnosis of congenital toxoplasma, rubella, and cytomegalovirus infections. However, both false-positive and false-negative results have been noted. The presence of rheumatoid factor has been a major cause of false-positive results. Tests with high specificity include solid-phase IgM assays with antihuman IgM as a "capture" antibody and enzyme-linked antibody markers.

Nonspecific tests, such as quantitation of total IgM or IgA or detection of rheumatoid factor, have limited or no usefulness. Negative results do not rule out infection, and positive results must be regarded cautiously.

5. In fetal and neonatal infections, such as those caused by HIV, specific antibody testing is not usually helpful in establishing a diagnosis in the first 15 to 18 months of life. Tests for p24 antigenemia, blood culture, or polymerase chain reaction methods for viral nucleic acid detection are preferred and may need to be serially repeated if initially negative.

Culture, PCR, and antigen detection are preferred for HIV diagnosis

## CONCLUSION

Fetal and neonatal infections remain a highly significant and often frustrating challenge. They can be severe, and permanent sequelae are common. At the onset of infection, clinical signs and symptoms are often exceedingly subtle; thus, the physician must be quickly alerted to the infectious possibilities, particularly when specific treatment is available. Of all of these infections, the most preventable is rubella, and assurance of immunity before conception is a mandatory goal. Better control of the remainder may become possible in the future with newer bacterial and viral vaccines, better early diagnostic methods, and improved treatments.

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CHAPTER 70

# Sexually Transmitted Diseases

W. Lawrence Drew

With the emergence of acquired immunodeficiency syndrome (AIDS) in the 1980s, sexually transmitted diseases (STDs) received increased attention, although they have long been a major public health problem in all population groups and social strata. The most common agents are *Chlamydia trachomatis,* papillomavirus, herpes simplex virus, *Neisseria gonorrhoeae,* and the most worrisome, human immunodeficiency virus (HIV). Additional agents spread by sexual contact include hepatitis B, cytomegalovirus, syphilis, chancroid, and lymphogranuloma venereum. Table 70–1 lists the major sexually transmitted pathogens and the disease syndromes associated with them. These infections are discussed in detail in chapters related to the etiologic agents.

Depending on the pathogen, the disease produced may be local or systemic. For the localized STDs, due to chlamydia for example, the most common manifestations are inflammation (eg, urethritis, cervicitis), which may or may not be noticed by the patient. In some cases, deeper structures become involved when the infection spreads beyond the local site by direct extension (eg, epididymitis, salpingitis). As with other infectious diseases, some of these can gain access to the bloodstream and produce systemic symptoms and spread to other organs. The systemic STDs produce infection beyond the genital site as part of their basic pathogenesis (eg, HIV, hepatitis B, and syphilis); syphilis does and HIV and hepatitis B do not produce a local genital lesion. The most common clinical syndromes are discussed next.

Some STDs begin as localized infections; others are primarily systemic

## GENITAL ULCERS

Single or multiple ulcerative lesions on the genitalia are one of the most common manifestations of STDs. Infection may begin as a papule or pustule and evolve into an ulcer. Table 70–2 lists the major features of genital ulcerations. The nature of the ulcer and whether it is painful are significant differential features. The ulcer (chancre) of syphilis is typically single, firm and indurated but painless, whereas genital herpes ulcers are often multiple and quite painful. The evaluation of genital ulcers usually focuses on the separation of genital herpes, the most common cause in industrialized nations, and syphilis from other causes. In the laboratory workup, it should be emphasized that direct microscopy and serologic tests may be negative at the time of presentation of the syphilitic chancre and that cultures for herpes simplex virus are usually positive from vesicular, pustular, or ulcerative lesions but may be negative from crusted areas. Chancroid caused by *Haemophilus ducreyi,* relatively rare in the developed world, may be suggested by direct microscopy but requires a special selective medium for culture. Granuloma inguinale, a disease also seen primarily in developing countries, is characterized by chronic, persistent genital papules or ulcers. It is

Pain and induration are major differential features

*C. granulomatis* shows encapsulated Gram-negative bacilli on smear

TABLE 70–1

**Sexually Transmitted Agents and Diseases Caused**

| Agent | Disease or Syndrome |
|---|---|
| Bacteria | |
| *Neisseria gonorrhoeae* | Urethritis, cervicitis, proctitis, pharyngitis, conjunctivitis, endometritis, pelvic inflammatory disease, perihepatitis, bartholinitis, disseminated gonococcal infection |
| *Chlamydia trachomatis* | Nongonococcal urethritis, epididymitis, cervicitis, salpingitis, inclusion conjunctivitis, infant pneumonia, trachoma, lymphogranuloma venereum |
| *Ureaplasma urealyticum* | Nongonococcal urethritis |
| *Treponema pallidum* | Syphilis |
| *Haemophilus ducreyi* | Chancroid |
| *Calymmatobacterium granulomatis* | Granuloma inguinale |
| Viruses | |
| HIV | AIDS, AIDS-related complex (ARC), perinatal and congenital AIDS, aseptic meningitis, subacute neurologic syndromes, persistent generalized adenopathy, asymptomatic infection |
| Herpes simplex virus | Primary and recurrent genital herpes, aseptic meningitis, neonatal herpes |
| Papillomavirus | Condylomata accuminata, laryngeal papilloma of newborn, association with cervical carcinoma |
| Cytomegalovirus | Heterophil-negative infectious mononucleosis, congenital birth defects |
| Hepatitis B virus | Hepatitis B, acute and chronic infections |
| Molluscum contagiosum virus | Genital molluscum contagiosum |
| Protozoa | |
| *Trichomonas vaginalis* | Trichomonal vaginitis |
| Fungi | |
| *Candida albicans* | Vulvovaginitis, penile candidiasis |
| Ectoparasites | |
| *Phthirus pubis* | Pubic louse infestation |
| *Sarcoptes scabiei* | Scabies |

*Abbreviations:* AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.

caused by *Calymmatobacterium granulomatis,* an encapsulated Gram-negative bacillus, which has not been grown in artificial medium. The diagnosis is usually made by examination of Wright- or Giemsa-stained impression smears from biopsy specimens that demonstrate clusters of encapsulated coccobacilli in the cytoplasm of mononuclear cells.

## GENITAL WARTS

Many genotypes of papillomaviruses

Some types associated with carcinoma of the cervix

Genital warts may be caused by human papillomavirus (condyloma acuminatum) or *Treponema pallidum* (condyloma latum). There are over 70 genotypes of human papillomavirus (HPV), of which types 6, 11, 16, 18, and 32 are the predominant causes of genital warts. In women, HPV types 16, 18, and 31 are usually associated with flat or subclinical warts and are the viral types that may be associated with cervical dysplasias, carcinoma in situ, and invasive cervical cancer. Condylomata lata are painless mucosal warty erosions that develop in warm, moist sites such as the genitals and perineum in about one third of cases of secondary syphilis. Darkfield examinations are invariably positive as are both nontreponemal and treponemal serologic tests.

## URETHRITIS

Urethritis usually manifests as dysuria, urethral discharge, or both. The discharge may be prominent enough to be the chief complaint or may have to be milked from the urethra.

TABLE 70–2

**Causes of Genital Ulcerations**

| Disease | Type of Lesion | Type of Inguinal Adenopathy[a] | Diagnosis |
|---|---|---|---|
| Genital herpes | Multiple grouped vesicles to coalesced ulcers, painful | Tender, discrete, nonsuppurative | Culture, enzyme immunoassay |
| Chancroid | Tender, shallow, painful ulcer, not indurated ulcer | Suppurative | Culture |
| Syphilis | Nontender, indurated ulcer | Rubbery consistency | Darkfield exam, serology |
| Lymphogranuloma venereum | Painless, small ulcer or papule, usually healed at time of presentation | Discrete progressing to suppurative, draining fistulas | Culture, serology |
| Granuloma inguinale | Papular to nodular to ulcerative lesion(s), painless | "Pseudobubo" caused by induration of subcutaneous tissue in inguinal area | Giemsa stain of biopsy |

[a]Involvement of inguinal lymph nodes.

The major causes of urethritis are *N. gonorrhoeae* and *C. trachomatis,* followed by *Ureaplasma urealyticum* and herpes simplex virus. Infection with more than one organism is common, particularly dual gonococcal and chlamydial infection. Up to 20% of cases have no established etiology but are probably infectious.

*C. trachomatis* and *N. gonorrhoeae* often coinfect

The diagnosis of gonorrhea is established primarily by culture, although direct examinations (Gram stain, DNA assays) may suffice in symptomatic patients. DNA-based assays are comparable to culture for screening. Newly developed nonculture techniques (eg, DNA amplification) are superior to culture for *C. trachomatis*, while culture is the most appropriate test for herpes simplex virus. Treatment depends on the etiologic agent and whether the disease has progressed beyond the local site. Empiric regimens are directed at the two most common causes, *N. gonorrhoeae* and *C. trachomatis.* In cases of gonorrhea, concurrent treatment for chlamydia is recommended, unless the latter has been specifically excluded. In general, the same approach is followed for epididymitis and cervicitis.

Culture and DNA-based assays available

Combined treatment often recommended

## EPIDIDYMITIS

Unilateral swelling of the epididymis is a common clinical illness seen in sexually active men. It is usually quite painful, with fever and acute unilateral swelling of the testicle that is sometimes confused with testicular torsion. In the preantibiotic era, approximately 10 to 15% of untreated gonococcal infections resulted in epididymitis. In developed countries, the two most common causes of epididymitis are *N. gonorrhoeae* and *C. trachomatis,* especially in younger men. In men older than 35 and in homosexual men, Enterobacteriaceae and coagulase-negative staphylococci may also cause the disease, probably from reflux of infected urine into the epididymis. Treatment depends on demonstration of the etiologic agent in urethral specimens or epididymal aspirates (see treatment of urethritis for additional considerations).

Gonoccoccal and chlamydial infections more common in men 35 years and younger

Enterobacteriaceae and *S. epidermidis* more common in older men

## CERVICITIS

Gonococcal, chlamydial, and herpes simplex virus infections most common

The microbial etiology of cervical infections is varied; *N. gonorrhoeae* and *C. trachomatis* cause endocervicitis, and herpes simplex virus can infect the stratified squamous epithelium of the ectocervix. The major clinical manifestation of cervicitis is a mucopurulent vaginal discharge. The cervix is friable and inflamed, and polymorphonuclear leukocytes are present in the exudate. Chlamydial, gonococcal, and viral cultures are needed to demonstrate the etiologic agent. Therapy depends on the etiologic agent involved (see treatment of urethritis for additional considerations).

## VAGINITIS AND VAGINAL DISCHARGE

Pelvic examination helps define important infection sites

Symptomatic vaginal discharge may occur alone or accompany salpingitis, endometritis, and cervicitis. Evaluation includes pelvic examination, cervical cultures for *N. gonorrhoeae* and *C. trachomatis,* and microscopic examination of the discharge. Measurement of the pH of the discharge may also be helpful. Pelvic examination is valuable in determining whether uterine, adnexal, or cervical tenderness is present and whether the source of the discharge is the cervix or the vagina.

*Candida* vaginitis causes itching, thick discharge

*Trichomonas* infection produces foamy discharge

The clinical and laboratory findings vary with the etiologic agent. *Candida albicans* generally produces a vulvovaginitis associated with pruritus and erythema of the vulvar area and a discharge with the consistency of cottage cheese. Microscopic demonstration of yeast and pseudomycelia in a potassium hydroxide or Gram stain preparation of the exudate confirms the diagnosis. *Trichomonas vaginalis* typically produces a foamy, purulent vaginal discharge. The pH is variable (usually >5.0), and numerous polymorphonuclear cells and motile trichomonads are seen on wet mount examination.

Bacterial vaginosis a shift in flora with anaerobic overgrowth

KOH added to discharge produces a fishy smell (amines)

Clue cells are present, and lactobacilli are absent

Bacterial vaginosis (BV), previously termed "nonspecific vaginitis," is the most common form of vaginitis in women. BV is associated with overgrowth of multiple members of the vaginal anaerobic flora, genital mycoplasmas, and a small Gram-negative rod (*Gardnerella vaginalis*), once believed to be the sole cause of the disease. The vaginal discharge of BV is yellowish, homogenous, and adherent to the vaginal wall. The pH is greater than 5.0. Addition of KOH to the vaginal secretions produces a fishy smell as a result of volatilization of amines. The Gram stain shows a shift from the usual lactobacillary flora to one of many Gram-negative coccobacilli. Clue cells, which are vaginal epithelial cells heavily coated with *G. vaginalis,* may also be seen. Therapy depends on the etiologic agent.

## PELVIC INFLAMMATORY DISEASE

Multiple etiologic agents; gonococcus predominant

Incidence higher with use of intrauterine devices

Clinical manifestations of pelvic inflammatory disease (PID) vary but generally include lower abdominal pain elicited by movement of the cervix or palpation of the adnexal or endometrial areas. About 50% of cases are caused by *N. gonorrhoeae.* Nongonococcal PID has a complex and sometimes polymicrobial etiology, including *C. trachomatis, Bacteroides,* anaerobic streptococci, and *Mycoplasma hominis* alone or in various combinations. In general, nongonococcal PID is milder than that associated with *N. gonorrhoeae* infection. The incidence of PID is five to ten times higher in women with intrauterine devices than in those not using this form of contraception. The diagnosis is established most reliably by culture of peritoneal aspirates from the vaginal cul-de-sac. Treatment of PID is complex because of the multiple etiologies and relative inaccessibility of the definitive diagnostic specimen.

## LYMPHADENITIS

Generalized adenopathy in secondary syphilis

Inguinal lymphadenitis may be seen with several STDs, especially primary herpes simplex infection and lymphogranuloma venereum. The latter is caused by specific strains of *C. trachomatis.* It may begin as a small genital ulcer, which is frequently unnoticed. More often, the first evidence of lymphogranuloma venereum is a tender swollen inguinal lymphadenitis, which may suppurate and drain spontaneously if not treated. Primary syphilis may be associated with unilateral or bilateral inguinal lymph node enlargement, but these nodes are not usually tender. Secondary syphilis may be associated with generalized lymphadenopathy.

## SYSTEMIC SYNDROMES

As indicated earlier, some STDs may manifest important pathology outside the genital tract, including diseases such as syphilis, hepatitis B, and AIDS whose most devastating consequences are at nongenital sites. These diseases can be highly complex, involving multiple organs and life-long illness. These organisms and diseases are best reviewed by referring back to the specific chapters that deal with each agent.

Most serious effects of syphilis, hepatitis B, and AIDS are outside of the genital tract

## ADDITIONAL READING

Centers For Disease Control and Prevention. Sexually transmitted diseases treatment guidelines—2002. *MMWR Morb Mortal Wkly Rep* 2002;51(RR06):1–80. This is a thorough guide to the recognition and current treatment recommendations for all STDs.

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CHAPTER 71

# Infections in the Immunocompromised Patient

W. Lawrence Drew

Immunocompromised patients are those whose host defense mechanisms are impaired by an inherited deficit, disease, or treatment. The immunocompromised state increases the risk of infection with many of the common pathogens as well as with low-virulence organisms present in the normal flora or environment. The organisms involved are those most able to take advantage of situations such as disruption of the skin or mucosal barriers and the more specific immune defects, including (1) defects in the phagocytic response, (2) defects in the complement system, (3) defects in antibody-mediated immunity, (4) defects in cell-mediated immunity, and (5) loss of reticuloendothelial function. Each of these defects tends to be associated with infections caused by specific groups of organisms (Table 71–1). For example, neutropenia and disorders of phagocytosis are associated with infections by Gram-positive cocci, Enterobacteriaceae, *Pseudomonas,* and fungi. In contrast, patients with defects in cell-mediated immunity tend to have severe viral, parasitic, and fungal infections or disease caused by bacteria that can multiply intracellularly (eg, mycobacteria). Those with defects in antibody production, such as agammaglobulinemia, are prone to infection with encapsulated organisms such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b.

Different types of immunocompromise are associated with different infecting organisms

## IMMUNE DEFICITS ASSOCIATED WITH INFECTION

### Defects in Epithelial Barriers

Defects in mucosal barriers represent an important prelude to infection by allowing organisms that normally colonize the skin, gastrointestinal tract, or upper airway access to deeper more vulnerable tissues. Burns, extensive trauma, and decubitus ulcers remove the epithelial defense of the skin; however, less obvious factors, such as cytotoxic therapy, may cause damage to mucosal surfaces that predisposes to attachment and replication of potentially pathogenic organisms and can cause loss of host-clearing mechanisms (eg, ciliary function). Defects in intestinal mucosal barriers are often associated with infections caused by Gram-negative aerobic and anaerobic enteric bacteria from the gut flora. Staphylococcal, streptococcal, and pneumococcal infections of the lung are particularly likely when the respiratory epithelium is damaged, whereas *Pseudomonas aeruginosa* infections are a common feature of severe burns.

Breaks in skin or mucosa provide entry

TABLE 71–1

**Infections in the Compromised Host**

| Type of Compromise | Example | Pathogen |
|---|---|---|
| ↓ Leukocyte number or function | Myelocytic leukemias<br>Chronic granulomatous disease<br>Granulocytopenia<br>Acidosis<br>Burns | Extracellular bacteria[a]<br>Opportunistic fungi |
| ↓ Humoral immune response | Lymphocytic leukemias<br>Multiple myeloma<br>Nephrotic syndrome<br>Antimetabolites<br>Hypogammaglobulinemia<br>AIDS | Encapsulated bacteria[b]<br>Enteroviruses<br>*Pneumocystis*<br>*Giardia*[c] |
| ↓ Complement components | Genetic deficiencies | Extracellular bacteria[a]<br>*Neisseria*[d] |
| ↓ Cellular immune response | AIDS<br>Hodgkin's disease<br>Transplantation<br>Steroids<br>Uremia<br>Antimetabolites<br>Malnutrition | *Pneumocystis*<br>Intracellular bacteria[e]<br>*Nocardia*<br>*Candida* and fungi of systemic mycoses<br>Viruses, especially herpesviruses<br>Protozoa[f]<br>*Strongyloides* |
| ↓ Reticuloendothelial system function | Splenectomy<br>Chronic hemolysis | *Pneumococcus*<br>*Salmonella*<br>*Listeria* |

[a] Bacteria that are unable to multiply in phagocytes.

[b] For example, *Streptococcus pneumoniae* and *Haemophilus influenzae* type b.

[c] Associated with lgA deficiency.

[d] Associated with C5, C6, C7, and C8 deficiencies.

[e] Bacteria capable of multiplying in unactivated macrophage.

[f] Includes *Toxoplasma* and *Cryptosporidium*.

## Defects in Number or Function of Phagocytes

When the natural barriers of the skin and mucosal surfaces are breached, the next major line of defense is the circulating phagocytes. To defend against infection, there must be an adequate number of these cells, and they must be able to move to the site of infection and ingest and kill invading organisms. Numerous defects in these processes have been described.

### *Neutropenia*

Neutropenia <500/mm$^3$ associated with infection; <100/mm$^3$ with bloodstream spread

Although normal neutrophil granulocyte counts vary greatly according to the age, sex, and race of the patient, the usual value is 2500 to 7500 cells/mm$^3$ of blood in adults. Neutropenia may result from inherited or acquired diseases, malignancies, use of cytotoxic drugs, or adverse reactions to therapeutic agents such as chloramphenicol. If the absolute neutrophil count decreases to fewer than 500 cells/mm$^3$, the incidence of infections increases markedly, and counts below 100 cells/mm$^3$ are associated with bacteremia. Immunocompromised patients differ in their ability to tolerate profound neutropenia. For example, patients with acquired immunodeficiency syndrome (AIDS) may not experience bacteremia as frequently as patients receiving chemotherapy. This may reflect damage to mucous membranes from the chemotherapy in addition to the neutropenia. Severe neutropenia is accompanied most frequently by bacterial infections caused by the pyogenic Gram-positive cocci, Enterobacteriaceae, *P. aeruginosa,* and *H. influenzae.* Fungal infections with *Candida, Aspergillus,* or the Zygomycetes are also common.

## *Defects in Chemotaxis and Leukocytic Function*

Defects in phagocytic defenses can be caused by multiple mechanisms that result in inadequate leukocyte chemotaxis or function (Table 71–2). Deficiencies of complement or immunoglobulins can decrease chemoattractants at the site of an infection, and certain metabolic diseases such as diabetes and uremia can alter the microenvironment of leukocytes to reduce their mobility and responsiveness to stimuli. This phenomenon has also been shown to occur in immune complex diseases such as lupus erythematosus. In each case, removal of the leukocyte to a normal environment restores its mobility and ability to respond chemotactically.

Diabetes and uremia can impair WBC function

Several genetic diseases produce specific defects in granulocyte bactericidal mechanisms that result in an immunocompromised host. Because they frequently diminish life span, these illnesses are usually seen in children. The most studied is chronic granulomatous disease, a group of inherited disorders of phagocytic cell superoxide production associated with frequent pyogenic infections, usually caused by catalase-positive organisms, such as *Staphylococcus aureus.* In Chédiak-Higashi disease, neutrophil lysosomes fail to fuse with the phagosome and the cells fail to destroy ingested organisms. These children also suffer recurrent infections with pyogenic organisms.

Chronic granulomatous disease due to lack of superoxide production

Chédiak-Higashi disease: failure of lysosome–phagosome fusion

The spectrum of infections in patients with phagocytic dysfunction is wide and includes repeated bouts of cellulitis, pharyngitis, perirectal and other abscesses, pneumonia, osteomyelitis, and bacteremia. Many pyogenic organisms other than staphylococci can be involved. Antimicrobic treatment given either therapeutically or prophylactically has helped greatly in the care of these patients, but they still suffer repeated bouts of infection that may ultimately prove fatal.

Prophylactic antimicrobics may be helpful

TABLE 71–2

### Disorders of Phagocytosis and Intracellular Phagocytic Killing

| | |
|---|---|
| **Chemotactic Defects** | **Ingestion** |
| Complement component deficiency | Actin–myosin dysfunction |
| Immunoglobulin deficiency | Drugs (colchicine, tetracycline, cyclophosphamide) |
| Intrinsic defects | Hyperosmolar states |
| “Lazy leukocytes” | Acute infections |
| Leukocyte adhesion disorders | |
| Burns | |
| Hyperimmunoglobulin E syndrome (Job’s syndrome) | **Degranulation** |
| | Chédiak–Higashi syndrome |
| Collagen vascular disease | |
| Diabetes mellitus, uremia | **Killing** |
| | Lysosomal enzyme deficiency |
| **Opsonization** | Chronic granulomatous disease |
| Immunoglobulin deficiency | Glucose-6-phosphate dehydrogenase deficiency |
| Complement component deficiency | Drugs (phenylbutazone, chloramphenicol) |
| Interference by immune complexes (systemic lupus erythematosus) | |
| Sickle cell anemia | Glutathione reductase deficiency |

## Antibody Deficiency

IgA deficiency is associated with giardiasis

IgM and IgG deficiencies associated with infection by encapsulated organisms

Several congenital and acquired disorders can lead to inadequate synthesis of immunoglobulins as a result of deficiency or dysfunction of B lymphocytes. The most common and least serious is immunoglobulin A deficiency, which is associated with increased risk of gastrointestinal tract infection, especially with the parasite *Giardia lamblia.* Individuals with severe defects in IgG and IgM production (hypogammaglobulinemia or agammaglobulinemia) are prone to recurrent infections with encapsulated organisms such as *S. pneumoniae* or *H. influenzae,* which require opsonization for adequate phagocytosis. Sinusitis, otitis media, bacterial pneumonia, and bacteremia are the most common types of infection. Acquired deficiency in immunoglobulin production may occur in AIDS, multiple myeloma, non-Hodgkin's lymphoma, and certain types of chronic lymphocytic leukemia that involve monoclonal proliferation of one immunoglobulin-producing cell line and relative deficiencies of cells producing other antibodies. These patients are also prone to infections by systemically invasive organisms.

Immune serum globulin or some vaccines are useful

Repeated injections of immunoglobulins (immune serum globulin) may decrease the incidence and morbidity of infections in patients with hypo- or agammaglobulinemia. In those capable of some immune responses, the use of pneumococcal vaccine (Pneumovax) may provide a degree of protection against overwhelming infection with this organism.

## Complement Deficiency

Opsonization defects with C3 deficiency

Systemic *Neisseria* infection in C5–C8 deficiencies

Defects of the complement system also predispose patients to many infections. Individuals with deficiencies in C3 are prone to infections with encapsulated organisms that require opsonization and to a range of infections similar to those seen in patients with hypogammaglobulinemia. Those with deficiencies in later components in the complement sequence are prone to develop recurrent bacteremia caused by *Neisseria meningitidis* or *Neisseria gonorrhoeae* if they are infected with these species. Patients with defects in the early complement components, C1, C2, or C4, have less of a problem than those with later complement pathway deficiencies, because they retain the ability to use the alternative complement pathway to activate C3 and hence C5 to C9.

## Disorders in Cell-Mediated Immunity

CD4+ lymphocytes compromised in AIDS

Glucocorticoids have multiple effects on immune cell function

Both congenital and acquired abnormalities of the cell-mediated immune system occur. Congenital abnormalities, which are uncommon, include thymic dysplasia syndrome, ataxia telangiectasia, and severe combined immunodeficiency (both T- and B-cell deficiency). AIDS, now the most important cause of acquired cellular immunodeficiency, causes a depletion of CD4+ T lymphocytes. Another common source of acquired defects is seen especially in transplant recipients due to treatment with immunosuppressive or cytotoxic agents that damage both macrophage precursors and T lymphocytes. Cytotoxic chemotherapy for cancer with cyclophosphamide and other antimetabolites has these effects and also inhibits humoral immune responses. Glucocorticoids can have multiple effects, causing neutropenia, lymphopenia, and monocytopenia through suppression of cell production, inhibition of mobilization of neutrophils to the site of inflammation, and interference with cell-mediated immune responses through alteration of the responsiveness of monocytes and macrophages to lymphokines. In addition, glucocorticoids impair the function of cells lining the mucosal surfaces, thus increasing the chance of microbial invasion by this route. Combinations of glucocorticosteroids and immunosuppressive drugs are essential in the treatment of certain diseases but are particularly likely to interfere with the ability of a patient to combat new or established infections.

Defective cell-mediated immunity associated with many infections by intracellular microbes and herpesviruses

A detailed analysis of the infections associated with the different causes of cell-mediated and combined immune deficits is beyond the scope of this chapter. In general, defects in cell-mediated immunity are associated with increased susceptibility to infection with specific opportunistic pathogens, particularly facultative or obligate intracellular pathogens such as cytomegalovirus, fungi and mycobacteria (see Table 71–1). For example, infection with *Mycobacterium tuberculosis* and other mycobacteria in AIDS patients is an important clinical problem. Because of the wide range of potential infecting organisms, the sites of infection associated with defects in cell-mediated immunity are varied. These include superficial skin infections, lung infections, pharyngitis, otitis, sinusitis, bacteremia, retinitis, and abscesses. Simultaneous infections with multiple organisms are common.

## CLINICAL SITUATIONS ASSOCIATED WITH INFECTION

### Acquired Immunodeficiency Syndrome

The increasing worldwide prevalence and profound immunodeficiency of AIDS remains a major concern. Most patients die of human immunodeficiency virus infection per se or as a direct result of one of the opportunistic infections mentioned earlier. As a result of its importance, AIDS is discussed separately in Chapter 42.

AIDS is an extraordinary model of immunocompromise

### Malignancies

Although some malignancies compromise the immune system directly, the chemotherapeutic agents used to treat them are the primary cause of immunosuppression. In particular, the periods of granulocytopenia between the administration of high-dose chemotherapy and recovery of granulocyte-producing function are associated with infection. The organisms most common during this vulnerable period are generally the same as among the general population; for example, *Staphylococcus aureus* and *Escherichia coli,* but other pathogens such as *P. aeruginosa* and *Candida albicans* are more prominent than in the immunocompetent individual. As discussed earlier, chemotherapy may also compromise cell-mediated immunity, in which case infections due to intracellular bacteria and viruses are common. Finally, chemotherapy may damage mucosa (oral, intestinal, vaginal, rectal), allowing ingress of bacteria, fungi or viruses.

Chemotherapy of malignancy commonly decreases granulocytes, and causes mucosal damage

### Transplantation

Solid organ and bone marrow transplantations are among the most important advances in modern medicine. Their success depends to a great degree on the ability to control and manage the undesired aspects of the immunosuppressive regimens, primarily the susceptibility to infection as long as immunosuppression is used. The pattern of microorganisms varies with the type of transplant, as does the immunosuppressive therapy, but viruses are extremely important. Viruses of the herpesvirus family, such as herpes simplex, varicella–zoster, and cytomegalovirus are the most common, but respiratory syncytial virus and other respiratory viruses are also important. Bacteria associated with deficiencies in granulocytes and cell-mediated immunity are also involved; *Legionella* and *Nocardia* infections have been particularly prominent in kidney and heart transplant recipients and fungal infections are common. Recombinant granulocyte-macrophage colony-stimulating factor can accelerate the recovery of bone marrow myeloid elements in bone marrow transplant and some cancer chemotherapy patients, sometimes reducing the period of vulnerability.

Herpesviruses particularly common

*Legionella* and *Nocardia* in solid organ transplants

## DIAGNOSIS

Clinical recognition and treatment of infections in the immunocompromised patient are often difficult, because the infection may be relatively silent due to impairment of the immune response. Laboratory diagnosis can also be difficult, because many of the organisms involved require special culture media and grow slowly (Table 71–3); others such as *Pneumocystis carinii* cannot be grown at all. The increased involvement of low-virulence organisms commonly found in the normal flora may make it difficult to distinguish colonization from infection. Thus, isolation of *C. albicans* from the urine or the pharynx does not prove that it is the cause of a concurrent renal abscess or pneumonitis. Diagnostic procedures such as biopsy of involved organs are often needed to identify the causative agent.

Diagnosis often requires aggressive procedures

TABLE 71–3

**Agents Commonly Infecting Immunocompromised Patients**

| Agent | Decreased Phagocytosis | Complement Deficiencies | Hypo- or Agammaglobulinemia | Defects in Cell-Mediated Immunity |
|---|---|---|---|---|
| **Bacteria** | | | | |
| *Staphylococcus aureus* and β-hemolytic streptococci | +++[a] | ++ | ++ | |
| *Streptococcus pneumoniae* | +++ | + | +++ | |
| Enterobacteriaceae | +++ | + | + | |
| *Pseudomonas aeruginosa* | +++ | ++ | + | |
| *Haemophilus influenzae* | + | + | +++ | |
| *Salmonella* species | + | + | | +++ |
| *Listeria monocytogenes* | | | | +++ |
| *Mycobacterium* species | | | | +++ |
| *Legionella* | | | | +++ |
| *Nocardia asteroides* | | | | +++ |
| *Neisseria* species | | ++ | + | |
| **Fungi** | | | | |
| *Candida* species | | | | |
| Systemic | ++ | | | |
| Chronic mucocutaneous | | | | +++ |
| *Aspergillus* species | +++ | | | |
| *Phycomyces* species | +++ | | | |
| *Cryptococcus neoformans* | | | | +++ |
| *Coccidioides immitis* | | | | +++ |
| *Histoplasma capsulatum* | | | | +++ |
| *Pneumocystis carinii* | | | ++ | +++ |
| **Viruses** | | | | |
| Herpes simplex | | | + | +++ |
| Varicella–zoster | | | ++ | +++ |
| Cytomegalovirus | | | | +++ |
| Epstein–Barr | | | | +++ |
| Papovaviruses | | | | ++ |
| Respiratory syncytial virus | | | | +++ |
| Enteroviruses | | | +++ | |
| Hepatitis B | | | | +++ |
| Influenza | | | + | + |
| Adenoviruses | | | + | +++ |
| **Parasites** | | | | |
| *Giardia lamblia* | | | ++ | + |
| *Toxoplasma gondii* | | | | +++ |
| *Strongyloides stercoralis* | | | | +++ |
| *Cryptosporidium* | | | | +++ |

[a] Number of pluses indicates relative susceptibility to the organisms listed according to the immune deficits.

## TREATMENT

Successful treatment of infections in the compromised host depends on recognition of the deficit, early diagnosis, and prompt intervention. This requires identification of the organisms most likely to be involved in the infection. The index of suspicion must be very high, because the signs and symptoms of infection that are seen in immunocompetent individuals may be lacking. For example, in neutropenia the clinical signs of infection (eg, abscess formation) may not be apparent when the patient is first seen because of lack of reaction to the disease. It is thus usually necessary to initiate antimicrobic treatment before results of culture and antibiotic susceptibility tests are available. Broad-spectrum antimicrobic coverage is used initially and replaced with narrower spectrum agents, when the etiologic agent and its susceptibility are known, to reduce the risk of superinfection. In general, bactericidal antimicrobics are needed to control infections when host defenses are inadequate, and with severe infections a combination of synergistic agents may be necessary to provide increased bactericidal action.

Early diagnosis and treatment particularly important

Bactericidal antimicrobics required

Patients with neutropenia have high rates of infection, and mortality may be as high as 20 to 30% if bacteremia develops. Therefore, short-term prophylactic antibiotic treatment has been advocated for neutropenic patients and can be effective in preventing infection until the neutrophil count improves. Selection of resistant organisms and “breakthrough” bacteremia as a result of overwhelming infection are major risks of these strategies in these susceptible patients, and the physician must be alert to the possibility of superinfection with other pathogens during treatment.

Careful use of antimicrobic prophylaxis during neutropenic periods

Neutropenia can be ameliorated by the use of cytokines (eg, granulocyte-colony stimulating factor). There is increasing attention to prevention of opportunistic infections in patients disposed to them. For example, patients undergoing bone marrow transplantation may receive prophylactic acyclovir or ganciclovir to prevent herpesvirus and cytomegalovirus infection. AIDS patients receive prophylactic trimethoprim–sulfamethoxazole to prevent *P. carinii* pneumonia as well as toxoplasmosis.

Antiviral, antifungal, and antiparasitic prophylaxis selectively used

## ADDITIONAL READING

Dykewicz CA. Summary of the guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2001;33:139–144. This nicely summarizes a much larger, joint report that is also referenced for readers seeking even greater detail.

Fishman JA, Rubin RH. Infection in organ-transplant recipients. *N Engl J Med* 1998;338:1741–1751. Review of infectious disease complications in solid organ transplant recipients.

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CHAPTER 72

# Nosocomial Infections and Infection Control

KENNETH J. RYAN

"Nosocomial" is a medical term for "hospital-associated." Nosocomial infections are complications that arise during at least 5% of all hospitalizations. The morbidity, mortality, and costs associated with these infections is preventable to a substantial degree. The purpose of hospital infection control is prevention of nosocomial infections by application of epidemiologic concepts and methods.

## HISTORY: SEMMELWEIS AND CHILDBED FEVER

The shining example of the fundamental importance of epidemiology in detection and control of nosocomial infections is the work of Ignaz Semmelweis, which preceded the microbiologic discoveries of Pasteur and Koch by a decade. Semmelweis was assistant obstetrician at the Vienna General Hospital, where more than 7000 infants were delivered each year. Childbed fever (puerperal endometritis), which we now know is caused primarily by group A streptococci, was a major problem accounting for 600 to 800 maternal deaths per year. By careful review of hospital statistics between 1846 and 1849, Semmelweis clearly showed that the death rate in one of the two divisions of the hospital was 10 times that in the other. Division I, which had the high mortality, was the teaching unit in which all deliveries were by obstetricians and students. In division II, all deliveries were by midwives. No similar epidemic existed elsewhere in the city of Vienna, and mortality was very low in mothers delivering at home.

Childbed fever was associated with obstetricians on teaching unit

Midwife and home births had lower rates

Semmelweis postulated that the key difference between divisions I and II was participation of the physicians and students in autopsies. One or more cadavers were dissected daily, some from cases of childbed fever and other infections. Handwashing was perfunctory, and Semmelweis believed this allowed the transmission of "invisible cadaver particles" by direct contact between the mother and the physician's hands during examinations and delivery. In 1847, as a countermeasure, he required handwashing with a chlorine solution until the hands were slippery and the odor of the cadaver was gone. The results were dramatic. The full effect of the chlorine handwashing can be seen by comparing mortality in the two divisions for 1846 and 1848 (Table 72–1). The mortality in division I was reduced to that of division II, and both were below 2%.

Transmission from cadavers was suspected

Disinfectant handwashing reduced the infection rates

Unfortunately, because of his personality and failure to publish his work until 1860, Semmelweis' contribution was not generally appreciated in his lifetime. As his frustration mounted over lack of acceptance of his ideas, he became abusive and irrational, eventually alienating even his early supporters. Some believe that he also suffered from Alzheimer's disease. He died in an insane asylum in 1865, unaware that his concept of

TABLE 72–1

**Childbed Fever at the Vienna General Hospital**

| | Division I (Teaching Unit) | | | Division II (Midwife Unit) | | |
|---|---|---|---|---|---|---|
| Year | Births | Maternal Deaths | Percentage | Births | Maternal Deaths | Percentage |
| 1846[a] | 4010 | 459 | 11.4 | 3754 | 105 | 2.7 |
| 1848[b] | 3556 | 45 | 1.3 | 3219 | 43 | 1.3 |

[a] No handwashing.

[b] First full year of chlorine handwashing.

spread via direct contact would later be recognized as the most important mechanism of nosocomial infection and that handwashing would remain the most important means of infection control in hospitals.

## NOSOCOMIAL INFECTIONS AND THEIR SOURCES

Community infections are acquired before admission

Nosocomial infections are acquired in hospital

Infections occurring during any hospitalization are either community acquired or nosocomial. Community infections are those present or incubating at the time of hospital admission. All others are considered nosocomial. For example, a hospital case of chickenpox could be community acquired if it erupted on the fifth hospital day (incubating) or nosocomial if hospitalization was beyond the limits of the known incubation period (20 days). Infections appearing shortly after discharge (2 weeks) are considered nosocomial, although some could have been acquired at home. Infectious hazards are inherent to the hospital environment; it is there that the most seriously infected and most susceptible patients are housed and often cared for by the same staff.

Endogenous infections are part of hospital risk

The infectious agents responsible for nosocomial infections arise from various sources, including patients' own normal flora. In addition to any immunocompromising disease or therapy, the hospital may impose additional risks by treatments that breach the normal defense barriers. Surgery, urinary or intravenous catheters, and invasive diagnostic procedures all may provide normal flora with access to usually sterile sites. Infections in which the source of organisms is the hospital rather than the patient include those derived from hospital personnel, the environment, and medical equipment.

### Hospital Personnel

Cross-infection is usually by direct contact

Infected medical attendants are particularly dangerous

Infection from carriers can transmit to patients

Physicians, nurses, students, therapists, and any others who come in contact with the patient may transmit infection. Transmission from one patient to another is called **cross-infection.** The vehicle of transmission is most often the inadequately washed hands of a medical attendant. Another source is the infected medical attendant. Many hospital outbreaks have been traced to hospital personnel, particularly physicians, who continue to care for patients despite an overt infection. Transmission is usually by direct contact, although airborne transmission is also possible. A third source is the person who is not ill but is carrying a virulent strain. For *Staphylococcus aureus* and group A streptococci, nasal carriage is most important, but sites such as the perineum and anus have also been involved in outbreaks. An occult carrier is less often the source of nosocomial infection than a physician covering up a boil or a nurse minimizing "the flu." The carrier is difficult to detect unless the epidemic strain has distinctive characteristics or the epidemiologic circumstances point to a single person.

### Environment

Environmental contamination is relatively unimportant

The hospital air, walls, floors, linens, and the like are not sterile and thus could serve as a source of organisms causing nosocomial infections, but the importance of this route has generally been exaggerated. With the exception of the immediate vicinity of an infected individual or a carrier, transmission through the air or on fomites is much less important than that caused by personnel or equipment. Notable exceptions are when the environment becomes contaminated with *Mycobacterium tuberculosis* from a patient or *Legionella pneumophila* in the water supply. These events are most likely to result in disease when the organisms are numerous or the patient is particularly vulnerable (eg, after heart surgery or bone marrow transplant).

*M. tuberculosis* and *Legionella* are risks

## Medical Devices

Much of the success of modern medicine is related to medical devices that support or monitor basic body functions. By their very nature, devices such as catheters and respirators carry a risk of nosocomial infection, because they bypass normal defense barriers, providing microorganisms access to normally sterile fluids and tissues. Most of the recognized causes are bacterial or fungal. The risk of infection is related to the degree of debilitation of the patient and various factors concerning the design and management of the device. Any device that crosses the skin or a mucosal barrier allows flora in the patient or environment to gain access to deeper sites around the outside surface. Possible access inside the device (eg, in the lumen) adds another and sometimes greater risk. In some devices, such as urinary catheters, contamination is avoidable; in others, such as respirators, complete sterility is either impossible or impractical to achieve.

Equipment that crosses epithelial barriers provides microbial access

The risk of contamination leading to infection is increased if organisms that gain access can multiply within the system. The availability of water, nutrients, and a suitable temperature largely determine which organism will survive and multiply. Many of the Gram-negative rods such as *Pseudomonas, Acinetobacter,* and members of the Enterobacteriaceae can multiply in an environment containing water and little else. Gram-positive bacteria generally require more physiologic conditions.

Conditions for bacterial growth increase risk

Even with proper growth conditions, many hours are required before contaminating organisms become numerous. Detailed studies of catheters and similar devices show the risk of infection begins to increase after 24 to 48 hours and is cumulative even if the device is changed or disinfected at intervals. It is thus important to discontinue transcutaneous procedures as soon as medically indicated. The medical devices most frequently associated with nosocomial infections are listed below. The infectious risk of others can be estimated from the principles discussed previously. New devices are constantly being introduced into medical care, occasionally without adequate consideration of their potential to cause nosocomial infection.

Transcutaneous and indwelling devices should be changed as soon as possible

### *Urinary Catheters*

Urinary tract infection (UTI) accounts for 40 to 50% of all nosocomial infections, and at least 80% of these are associated with catheterization. The infectious risk of a single urinary catheterization has been estimated at 1%, and indwelling catheters carry a risk that may be as high as 10%. The major preventive measure is maintenance of a completely closed system through the use of valves and aspiration ports designed to prevent bacterial access to the inside of the catheter or collecting bag. Unfortunately, breaks in closed systems eventually occur if the system is in place for more than 30 days. The urine itself serves as an excellent culture medium once bacteria gain access. Although *Escherichia coli* is still a leading cause of nosocomial UTIs, other Enterobacteriaceae and *Pseudomonas* are more likely than in the community setting.

Closed urinary drainage systems are still violated

*E. coli* and other Gram-negative bacteria predominate

### *Vascular Catheters*

Needles and plastic catheters placed in veins (or, less often, in arteries) for fluid administration, monitoring vital functions, or diagnostic procedures are a leading cause of nosocomial bacteremia. These sites should always be suspected as a source of organisms whenever blood cultures are positive with no apparent primary site for the bacteremia. Contamination at the insertion site is generally staphylococcal, with continued growth in the catheter tip. Organisms may gain access somewhere in the lines, valves, bags, or

Skin is primary source for intravenous contamination bottles of intravenous solutions proximal to the insertion site. The latter circumstance usually involves Gram-negative rods. Preventive measures include aseptic insertion technique and appropriate care of the lines, including changes at regular intervals.

### *Respirators*

Changing controls nebulizer contamination

Machines that assist or control respiration by pumping air directly into the trachea have a great potential for nosocomial pneumonia if the aerosol they deliver becomes contaminated. Bacterial growth is significant only in the parts of the system that contain water; in systems using nebulizers, bacteria can be suspended in water droplets small enough to reach the alveoli. The organisms involved include *Pseudomonas,* Enterobacteriaceae, and a wide variety of environmental bacteria such as *Acinetobacter.* The primary control measure is periodic changing and disinfection of the tubing, reservoirs, and nebulizer jets.

## Blood and Blood Products

Risk of hepatitis B, hepatitis C, and HIV is related to blood manipulation

Screen is determined by institutional policy

Infections related to contact with blood and blood products are generally a risk for health care workers rather than patients. Manipulations ranging from phlebotomy and hemodialysis to surgery carry varying risk of blood containing an infectious agent reaching mucous membranes or skin of the health care worker. The major agents transmitted in this manner are hepatitis B, hepatitis C, and human immunodeficiency virus (HIV). Control requires meticulous attention to procedures that prevent direct contact with blood, such as the use of gloves, eyewear, and gowns. Cuts and needle sticks among health care workers carry a risk approaching 2%. Identification of hepatitis virus and HIV carriers is a part of a protective process that must be balanced by patient privacy considerations. Health care facilities all have established policies concerning serologic surveillance of patients and the procedures to follow (eg, testing, prophylaxis) when blood-related accidents occur. Similarly, products for transfusion undergo extensive screening in order to protect the recipient.

# INFECTION CONTROL

Antisepsis attacks contaminating organisms

Asepsis prevents contamination

Infection control is the sum of all the means used to prevent nosocomial infections. Historically, such methods have been developed as an integral part of the study of infectious diseases, often serving as key elements in the proof of infectious etiology. Semmelweis' handwashing is the first example. Later in the 19th century, Joseph Lister achieved a dramatic reduction in surgical wound infections by infusion of a phenolic antiseptic into wounds. This local destruction of organisms was known as **antisepsis,** and it sometimes included liberal applications of disinfectants, including sprays to the environment. As it became recognized that contamination of wounds was not inevitable, the emphasis gradually shifted to preventing contact between microorganisms and susceptible sites, a concept called **asepsis.** Asepsis, which combines containment with the methods of sterilization and disinfection discussed in Chapter 11, is the central concept of infection control. The measures taken to achieve asepsis vary, depending on whether the circumstances and environment are most similar to the operating room, hospital ward, or outpatient clinic.

## Asepsis

### *Operating Room*

Sterile drapes and instruments prevent contact of organisms with wound

The surgical suite and operating room represent the most controlled and rigid application of aseptic principles. The procedure begins with the use of an antiseptic scrub of the skin over the operative site and the hands and forearms of all who will have contact with the patient. The use of sterile drapes, gowns, and instruments serves to prevent spread through direct contact, and caps and face masks reduce airborne spread from personnel to the wound. As all students learn the first time they scrub, even the manner of dressing and moving in the operating room are rigidly specified, and those involved assume a strict aseptic attitude as well as their masks and gowns. In some hospitals, the air entering the operating room is filter sterilized, but this practice is expensive and its value unproved. The level of bacteria in the air is generally more related to the number of persons and amount of movement in the operating room than to incoming air. The net effect of these procedures is to draw a sterile curtain around the operative site, thus minimizing contact with microorganisms. Surgical asepsis is also used in other areas where invasive special procedures such as cardiac catheterization are performed.

Airborne bacteria are associated with personnel in operating room

### *Hospital Ward*

Although theoretically desirable, strict aseptic procedures as used in the operating room are impractical in the ward setting. Asepsis is practiced by the use of sterile needles, medications, dressings, and other items that could serve as transmission vehicles if contaminated. A "no touch" technique for examining wounds and changing dressings eliminates direct contact with any nonsterile item. Invasive procedures such as catheter insertion and lumbar punctures are performed under aseptic precautions similar to those used in the operating room. In all circumstances, handwashing between patient contacts is the single most important aseptic precaution.

Handwashing is the most important measure

### *Outpatient Clinic*

The general, aseptic practices used on the hospital ward are also appropriate to the outpatient situation as preventive measures. The potential for cross-infection in the clinic or waiting room is obvious but has been little studied regarding preventive measures. Patients who may be infected should be segregated whenever possible using techniques similar to those of hospital ward isolation. The examining room may be used in a manner analogous to the private rooms on a hospital ward. Although this approach is difficult because of patient turnover, it should be attempted for infections that would require strict or respiratory isolation in the hospital.

Waiting areas present a risk

## Isolation Procedures

Patients with infections pose special problems, because they may transmit their infections to other patients either directly or by contact with a staff member. This additional risk is managed by the techniques of isolation, which place barriers between the infected patient and others on the ward. Because not every infected patient presents with suspect signs and/or symptoms, some precaution should be taken with all patients. In the system recommended by the Centers for Disease Control and Prevention, these are called **standard precautions** and include the use of gowns and gloves when in contact with patient blood or secretions. These are particularly directed at protecting health care workers from HIV and hepatitis infection. For those with suspect or proven infection, additional precautions are taken, the nature of which is determined by the known mode of transmission of the organism. These **transmission-based precautions** are divided into those directed at airborne, droplet, and contact routes. The **airborne** transmission precautions are for infections known to be transmitted by extremely small ($<5\ \mu m$) particles suspended in the air. This requires that the room air circulation be maintained with negative pressure relative to the surrounding area and be exhausted to the outside. Those entering the room must wear surgical masks, and in the case of tuberculosis, specially designed respirators. **Droplet** precautions are for infections where the organisms are suspended in larger droplets, which may be airborne, but generally do not travel more than 3 feet from the patient who generates them. These can be contained by the use of gowns, gloves, and masks when working close to the patient. **Contact** precautions are used for infections that require direct contact with organisms on or pass in secretions of the patient. Diarrheal infections are of special concern because of the extent to which they contaminate the environment. Details of the precautions and examples of the typical infectious agents are summarized in Table 72–2.

Standard precautions protect health care workers from HIV

Transmission precautions block airborne, droplet, and contact routes

TABLE 72–2

**Precautions for Prevention of Nosocomial Infections**

| | Elements | | | | | |
|---|---|---|---|---|---|---|
| Precaution | Room | Handwashing[a] | Gloves | Gowns | Mask[b] | Typical Diseases |
| Standard | | After removing gloves, between patients | Blood, fluid contact | Blood, fluid contact | | All |
| Transmission-based | | | | | | |
| Airborne | Private, negative pressure[c] | After removing gloves, between patients | Room entry | Room entry | Room entry or respirator[d] | Measles, chickenpox, tuberculosis[d] |
| Droplet | Private[e] | After removing gloves, between patients | Blood, fluid contact | Blood, fluid contact | Within 3 feet of patient | Meningococcal meningitis, pertussis, plague, group A streptococcus, adenovirus, influenza, rubella |
| Contact | Private[e] | After removing gloves, between patients | Room entry | Patient contact | | Infectious diarrhea,[f] impetigo, *S. aureus* wounds, herpes, respiratory syncytial virus, parainfluenza virus, scabies |

[a] Using a disinfectant soap.

[b] Standard surgical mask.

[c] Room pressure must be negative in relation to surrounding area and the circulation exhausted outside the building.

[d] For patients with diagnosed or suspect tuberculosis, a specially filtered respirator/mask must be worn.

[e] Door may be left open and patients with the same organism may share a room.

[f] Particularly *Clostridium difficile, Escherichia coli* O:157, *Shigella* and incontinent patient shedding rotavirus or hepatitis A.

## Organization

Infection control programs determine and enforce policy

Modern hospitals are required to have formal infection control programs that include an infection control committee, epidemiology service, and educational activities. The infection control committee is composed of representatives of various medical, administrative, nursing, housekeeping, and support services. The committee establishes the institution's infection control procedures and regularly reviews information on the status of nosocomial infections in the hospital. When epidemiologic circumstances warrant it, the committee is empowered to take drastic action such as closing a hospital unit or suspending a physician's privileges.

Epidemiologic surveillance and outbreak investigation are required

The epidemiology service is the working arm of the infection control committee. Its functions are performed by one or more epidemiologists who usually have a nursing background. This work requires familiarity with clinical microbiology, epidemiology, infectious disease, and hospital procedures, as well as immense tact. The main activities are surveillance and outbreak investigation. Surveillance is the collection of data documenting the frequency and nature of nosocomial infections in the hospital to detect deviations from the institutional or national norms. Although routine microbiologic sampling of the hospital environment is of no value, programs to sample some of the medical devices known to be nosocomial hazards can be useful. On-the-spot investigation of potential outbreaks allows early implementation of preventive measures. This activity is probably the single most important function of the epidemiology service. Suspicion of an increased number of infections leads to an investigation to verify the facts, establish basic epidemiologic associations, and relate them to preventive measures. The primary concern is cross-infection, in which a virulent organism is being transmitted from patient to patient. Solution of the problem may require additional microbiologic investigations, such as bacteriophage typing of *S. aureus.*

## PREVENTION

The prevention of nosocomial infections is contingent on basic and applied knowledge drawn from all parts of this book. Applied with common sense, these principles can both prevent disease and reduce the costs of medical care.

## ADDITIONAL READING

Aitken C, Jeffries DJ. Nosocomial spread of viral diseases. *Clin Microbiol Rev* 2001;14:528–546. The viral component of nosocomial infections is often neglected. This review discusses viral agents by their route of transmission in the health care setting including the blood-borne viruses.

Fourth Decennial Conference on Healthcare-associated Infections. *Emerg Infect Dis* 2001;7:169–368. This special issue contains state-of-the-art articles on all the current topics in nosocomial infection. The cover reproduces a painting titled "Semmelweis: Defender of Motherhood."

# Glossary

# Glossary

The glossary is intended as an adjunct to the index for rapid reference. It includes words and phrases that have not been defined in the text or that have been defined but are used frequently in later chapters. Where a word has multiple uses, the one relevant to this text is emphasized.

The prefixes and suffixes in each alphabetical section include word elements used in combined form. The meaning of many words can be derived from the prefixes and suffixes and therefore have not been included in the glossary.

**A-, An-** Without.
**Acanthosis** Hyperplasia and thickening of prickle cell layer of skin.
**Accessory sinuses** Blind-ended cavities in bone draining into nasal cavity.
**Achlorhydria** Absence of hydrochloric acid in stomach.
**Acid fast** Describes an organism that resists acid decolorization after straining.
**Acidosis** Increased acidity of body fluid.
**Aciduric** Resistant to effects of acid.
**Actin** Major structural protein of the eukaryotic cell cytoskeleton.
**Addison's disease** Result of primary deficiency of production of adrenal hormones.
**Adenocarcinoma** Malignant tumor derived from glandular epithelium.
**Adhesin** Surface component of a microbe that binds to a cell receptor.
**Adnexa (uterine)** Fallopian tubes and ovaries.
**Adrenal** Important endocrine glands situated above the kidneys.
**Aerobactin** A hydroxamate siderophore produced by many bacteria.
**Agammaglobulinemia** Absence of immunoglobulins in the blood.
**Agglutinate** Clumping.
**Agranulocytosis** Failure of white blood cell production in bone marrow.
**-algia** Pain.
**Allele** Alternate forms of a gene at the same chromosomal locus.
**Alloantigen** An antigen that exists in alternate allelic forms.
**Allosteric** Property of a protein that leads to a change in conformation and function associated with attachment of a smaller effector molecule.
**Alveoli (lung)** Microscopic air sacs in lung.
**Ameboma** A local inflammatory mass caused by an amebal infection.
**Amniotic fluid** Fluid in amniotic sac surrounding the fetus.
**Anaerobe** Microorganism that multiplies only in the absence of oxygen.
**Analog** Structurally or functionally similar substance or property.
**Anamnestic** Enhanced immunological memory response on reexposure to antigen.
**Anaphylaxis** Immediate and severe antibody-mediated hypersensitivity reaction.
**Anergic** Absence of ability to respond to antigen.
**Aneurysm** Localized abnormal dilatation of blood vessel.
**Anicteric** Absence of clinical jaundice.
**Anneal** Subject to controlled heating and cooling to achieve a particular property.
**Anorexia** Loss of appetite.
**Anoxia** Lack of adequate oxygenation of blood or tissues.
**Anterior horn cell** Motor neuron in the anterior gray matter of the spinal cord.

**Anthropo-** Relationship to humans.
**Antibiogram** Pattern of in vitro susceptibilities to different antimicrobics.
**Antibody** An immunoglobulin molecule that interacts with the antigen that elicited its production.
**Antigen** A substance that elicits a specific immunological response or reacts with antibody in vitro. (See Immunogen *and* Hapten).
**Antiserum** Serum containing specific antibodies.
**Antitoxin** An antibody that neutralizes an exotoxin.
**Antitussive** Substance that helps control coughing.
**Aphonia** Loss of speech.
**Aplastic anemia** Failure of red cell production in bone marrow.
**Apnea** Temporary absence of breathing.
**Aqueduct of Sylvius** Canal connecting the third and fourth ventricles of the brain.
**Arachidonic acid** Precursor of prostaglandins.
**Arachnoid** The middle of three membranes that cover the brain and spinal cord (meninges).
**Arrythmia** Irregularity of heartbeat.
**Arteriole** Smallest artery leading to capillary.
**Arthralgia** Pain in a joint.
**Arthro-** Pertaining to joints.
**Aryepiglottis** Related to the epiglottis and the arytenoid cartilage.
**Ascites** Fluid in a peritoneal cavity.
**Ascus** A sac. In mycology, a specialized structure containing spores termed ascospores.
**Asepsis** Exclusion of pathogenic organisms.
**Asphyxia** Suffocation.
**Astrocyte** Connective tissue cell of the central nervous system.
**Ataxia** Disturbance of muscular coordination.
**Ataxia telangiectasia** Hereditary disorder causing ataxia and permanent dilatation of some blood vessels.
**Atelectasis** Collapse of part of lung.
**Atherosclerosis** Hardening of the arteries.
**Atrophy** Wasting.
**Attenuated** Reduced in virulence, (eg, organisms in a live vaccine).
**Auto-** Self, or arising from within.
**Autochthonous flora** Organism with intimate and permanent association with an epithelial surface.
**Autoimmunity** An immune response against the body's own tissues.
**Autolysis** Lysis of a cell by its own enzymes.
**Autonomic** Relates to involuntary nervous system controlling cardiac, vascular, intestinal, and other functions.
**Auxo-** Pertaining to growth.
**Auxotroph** Bacterial mutant that has lost the ability to synthesize an essential nutrient or metabolite.
**Avascular** Absence of blood vessels or blood supply.
**Axenic** Refers to pure cultures of a microorganism without presence of a contaminating or symbiotic organism.
**Axon** The extension of a neuron that conducts nerve impulses.

**Bacteremia** Bacteria in the blood.
**Bacteriocins** Proteins produced by one bacterium that kill another of the same or other species.
**Bacteriophage** Bacterial virus.
**Bacteriostasis** Inhibition of bacterial growth without killing.
**Bacteriuria** Bacteria in the urine.
**Bartholin's glands** Lubricating glands on either side of the vaginal opening.
**Basophil** Polymorphonuclear leucocyte with basophilic granules.
**Basophilic** Stains with a basic dye.
**Biliary** Pertaining to the bile and bile ducts.
**Bilirubin** A bile pigment.
**Bio-** Pertaining to life.
**Biotype** Subtype within a species characterized by physiologic properties.
**-blast** Precursor cell.
**Bleb** See Bulla.
**Blepharal** Pertaining to the eyelids.
**Blepharo-** Pertaining to the eyelid.
**Blepharoplast** Basal body of a cilium or flagellum.
**Blood–brain barrier** Functional barrier preventing passage of large molecules to the brain parenchyma.
**Bolus** Rounded mass that may obstruct (eg, fecal bolus) or a concentrated mass (eg, an antibiotic) given rapidly and intravenously.
**Bothria** Paired sucking grooves in the head of the fish tapeworm (*Diphyllobothrium*).
**Brady-** Slowing.
**Bradycardia** Unusually slow heartbeat.
**Bronchial tree** Bronchi and bronchioles that conduct gases to and from the lung alveoli.
**Bronchiectasis** Pathological dilatation of terminal bronchi.
**Bronchiole** Smallest subdivision of bronchial tree.
**Broncho-** Pertaining to the bronchial tree.
**Bubo** Swollen, inflamed, infected lymph node.
**Buccal** Pertaining to the cheek.
**Bulla** Blister or vesicle containing semipurulent fluid.
**Bursa** Sac filled with fluid (eg, protecting a joint or tendon).

**Calculus** Pathological stone (eg, renal or gallbladder calculus).
**Calmodulin** A protein present in eukaryotic cells that activates some essential enzymes when it has bound calcium.
**Capillary** The smallest blood vessel connecting the arterial and venous systems.
**Capsid** The outer protein coat of a virus that protects its nucleic acid.
**Capsomeres** Subunits of viral capsids.
**Carbuncle** A necrotic staphylococcal infection of skin and subcutaneous tissue that has spread from infected furuncles.
**Carcinoma** Malignant growth of epithelial cells.

**Cardio-** Pertaining to the heart.
**Cardiolipin** A phospholipid occurring naturally in mitochondrial membranes against which antibodies are formed in syphilitic infection.
**Cardiomyopathy** Disease of heart muscle.
**Caseous** Cheesy in consistency.
**Catalase** Enzyme that catalyzes the reduction of toxic hydrogen peroxide to oxygen and water.
**Cell-mediated immunity** Immune reactions in which T lymphocytes play the pivotal role.
**Cellulitis** Inflammation of subcutaneous tissue.
**Cementum** Layer of modified bone on tooth root.
**Cerebrospinal fluid** Fluid that fills spaces within and surrounding the central nervous system.
**Cervical** Pertaining to the neck or uterine cervix.
**Cervix** The constricted portion of an organ. Usually refers to the lower part of the uterus.
**Chancre** Sore or ulcer that develops at the site of an infection. Most often used to describe the primary syphilitic lesion.
**Chelator** Compound that binds metallic ions.
**Chemoprophylaxis** Use of antimicrobics to prevent infection.
**Chemotaxis** Attraction of a motile cell to a chemical.
**Chitin** Polysaccharide forming exoskeletons of some insects or cell walls of some fungi.
**Cholangitis** Inflammation of the bile ducts.
**Chole-** Pertaining to bile.
**Cholecystitis** Inflammation of the gallbladder.
**Cholestasis** Interruption of the flow of bile.
**Cholinergic nerves** Nerve fibers that release acetylcholine as a mediator at their effector terminals.
**Chordae tendinae** Small tendons that connect papillary muscles of the heart to the cusps of the atrioventricular valves.
**Chorea** Rapid purposeless involuntary movements.
**Chorioallantoic membrane** The outer membrane surrounding an avian embryo within the egg shell.
**Chorionic membrane** The outer extraembryonic membrane from which the placenta originates.
**Chorioretinitis** Inflammation of choroid and retina of the eye.
**Choroid plexus** Vascular invagination into the cerebral ventricles. Produces the cerebrospinal fluid.
**Chromatin** Complex of DNA and histones making up the chromosomes of eukaryotic cells.
**Chronic granulomatous disease** Genetic disorder causing absence of $H_2O_2$ production and myeloperoxidase activity of phagocytes. Results in repeated infections with catalase positive bacteria.
**-cidal** Killing.
**CIE** See Counterimmunoelectrophoresis.
**Cilia** Surface structures of some eukaryotic cells that beat rhythmically to move mucus over surfaces or confer motility on some single-celled organisms.
**Cirrhosis** Fibrosis and nodular regeneration of the liver with loss of function.
**Cistron** The smallest functional genetic unit. A gene.
**Clone** Identical progeny of a single cell, gene, or genes.
**CMI** See Cell-mediated immunity.
**Co-agglutination** Agglutination involving two organisms, one of which acts as an inert particle coated with specific antibody to the other.
**Co-cultivation** Process that can be used for unmasking latent virus by growing susceptible cells with those from affected tissue.
**Coarctation** Stricture or narrowing (eg, of the aorta).
**Codon** The three nucleotides encoding an amino acid or a chain termination signal.
**Collagen** Fibrous component of connective tissue.
**Coloboma** A defect of the eye.
**Colostrum** Initial secretion of the breast after delivery (contains antibodies and lymphocytes).
**Comedo** Blocked sebaceous duct with retention of sebum (blackhead).
**Commensal** Organism of the normal flora that has a symbiotic relationship with the host.
**Complement** A system of serum proteins that act in sequence to mediate inflammatory and some immune responses.
**Condyloma acuminatum** A wart-like infectious benign growth that occurs on the genitalia and in the anal canal.
**Conidia** Asexual fungal reproductive spore-like bodies.
**Conidiophore** Fungal structure that bears conidia.
**Copepod** Minute fresh water fleas that serve as intermediate hosts for some parasites.
**Coprolith** Stony, hard stool.
**Coracidium** The ciliated free swimming embryo of certain tapeworms.
**Cornea** Clear, anterior portion of the eyeball.
**Cortex** The outer layer of an organ.
**Corticosteroid** Steroid hormone from adrenal gland; some are anti-inflammatory.
**Coryza** Catarrhal rhinitis (eg, from the common cold).
**Counterimmunoelectrophoresis** A technique for increasing the sensitivity and speed of the immunodiffusion procedure by the application of an electrophoretic field (see Immunodiffusion).
**Crepitation** A crackling or rattling sound.
**Cribriform plate** Area of bone above nasal cavity through which pass the olfactory nerves.
**Croup** Manifestations of laryngeal obstruction from inflammation or other causes.
**Crustacean** Hard shelled invertebrates such as crabs, shrimp, and lobsters.
**CSF** *See* Cerebrospinal fluid.
**Curare** A plant extract that produces generalized paralysis by acting at neuromuscular junctions.
**Cuticle** Skin or surface layer.
**Cyanosis** Blue color of skin caused by lack of oxygen.
**Cystic fibrosis** Congenital disease of secreting glands affecting pancreas, respiratory tract, and sweat glands. Associated with viscid respiratory mucus and chronic respiratory infections.

**Cysticercus** Larval form of tapeworm enclosed in a cyst.
**Cysto-** Pertaining to the bladder.
**Cystoscope** Instrument for examining inside the urinary bladder.
**Cyto-** Pertaining to the cell.
**Cytokine** Hormone-like intercellular messenger molecule (eg, lymphokine and interleukin).
**Cytology** The study of cells rather than of tissues and organs.
**Cytoplasm** Cellular contents excluding the nucleus.
**Cytosol** Liquid portion of cytoplasm.
**Cytosome** The body of a cell apart from its nucleus.
**Cytostome** The mouth opening of certain ciliated protozoa.

**Dalton** Atomic mass unit that gives the same number as atomic weight.
**Debridement** Removing foreign matter and dead tissue.
**Decubitus ulcer** Pressure sore (bed sore).
**Defensins** A family of microbial, cationic, cystine rich polypeptides abundant in the azurophilic granules of polymorphonuclear leukocytes.
**Demyelination** Loss of nerve sheaths.
**Dendritic** Branched.
**Dermatophyte** Fungus that causes skin infections.
**Dermis** Skin connective tissue immediately below the epidermis.
**Dermo-** Pertaining to the skin.
**Desquamation** Loss of skin epithelial cells.
**Dextran** A polymer of D-glucose.
**Dimorphism** Occurring in two morphologic forms under different conditions.
**Diploid** Possessing two sets of chromosomes.
**Disseminated intravascular coagulation (DIC)** A clinical syndrome with multiple causes. Thrombocytopenia and complex coagulation abnormalities are prominent.
**Diverticulum** Blind-ended extrusion from a hollow organ.
**Ductus arteriosus** Fetal blood vessel connecting the pulmonary artery to the descending aorta.
**Dys-** Difficult or painful.
**Dysentery** Pain and frequent defecation resulting from inflammation of the colon or other intestines, with blood and pus in the stool.
**Dyspareunia** Difficult or painful intercourse.
**Dysphagia** Difficulty in swallowing.
**Dysplasia** Histological evidence of possible premalignant changes in cells.
**Dyspnea** Shortness of breath.
**Dysuria** Difficult or painful urination.

**Ecchymosis** A large area of hemorrhage into the skin, often a coalescence of petechiae.
**Ecthyma** Eroded, scabbed lesion of the skin.
**Ecto-** Outside or outer.
**-ectomy** Surgical removal of.
**Ectopic pregnancy** Fetal development outside the uterus (usually in the fallopian tubes).
**Ectoplasm** Clear layer of cytoplasm near the cell membrane of amebas.
**Edema** Excessive fluid in tissues.
**EIA.** See Enzyme immunoassay.
**Elastosis** Disorder of fibroelastic proteins.
**Electrophoresis** Procedure for separating charged particles by differences in their migration in an electric field.
**ELISA** Enzyme-linked immunosorbent assay (*See* Enzyme immunoassay).
**Embolism** Sudden blockage of an artery.
**-emia** Of the blood.
**Emphysema (pulmonary)** Irreversible enlargement of alveolar sacs of lung.
**Empyema** Pus in a body cavity (eg, pleural cavity).
**Encephalitis** Inflammation of brain tissue.
**Endarteritis** Inflammation of the inner coat of an artery or arteriole.
**Endemic** A disease that is continuously present at subepidemic levels in a particular region, locality, or group.
**Endo-** Within.
**Endogenous** Originating within an organism.
**Endometrium** Interior epithelial lining of the uterus.
**Endonuclease** Enzyme of a class that hydrolyzes internal bonds of DNA or RNA. Involved in synthesis and breakdown of nucleic acids.
**Endophthalmitis** Inflammation of interior tissues of the eye.
**Endoplasm** Central portion of cytoplasm of cell.
**Endoplasmic reticulum** Ramifying membranes within the cytoplasm of eukaryotic cells.
**Endospore** Bacterial spore.
**Endotoxin lipid** A toxic moiety of bacterial cell wall lipopolysaccharide.
**Entactin** Protein component of the extracellular matrix.
**Enteric** Pertaining to the intestinal tract.
**Enteric fever** Typhoid or similar systemic *Salmonella* or *Yersinia* infection.
**Entero-** Pertaining to intestines.
**Enterobactin** A phenolate siderophore produced by *E. coli* and some other enteric species of bacteria.
**Enterochelin** Synonym for Enterobactin.
**Enucleation (ocular)** Removal of an eye intact.
**Enzootic** Disease present at low levels at all times in an animal community.
**Enzyme immunoassay** A method for detecting antigen–antibody reactions by labeling one of the reagents with detectable enzyme.
**Eosinophil** Polymorphonuclear leucocyte with eosinophilic granules.
**Epi-** Upon or additional to.
**Epicardium** Outer lining of the heart.
**Epidemic** A disease that rapidly affects many people in a circumscribed period of time.
**Epididymis** Tubular structure attached to the testes in which spermatozoa mature.
**Epigastrium** Upper central region of the abdomen overlying the stomach.

**Epiglottis** Movable structure overlying and protecting the larynx.
**Epiphysis** Growing end of bone.
**Episome** Plasmid or viral DNA that can replicate extrachromosomally or can integrate into chromosome.
**Epitope** Structural part of an antigen that determines specificity of an antigen–antibody reaction (also called antigenic determinant).
**Epitrochlear node** Lymph node above inner side of elbow.
**Erythema** Red color caused by dilatation of blood vessels.
**Erythema nodosum** Red raised skin nodules usually on the legs. Usually a manifestation of a hypersensitivity reaction.
**Erythro-** Red.
**Erythrocyte** Red blood cell.
**Eschar** Necrotic scab-like area of skin.
**Etiology** Cause of a disease.
**Eukaryote** Organism comprising one or more cells containing true nuclei.
**Eustachian tube** Tube connecting the middle ear and the nasopharynx.
**Exanthem** Disease in which skin rashes are major manifestations.
**Exocrine glands** Glands excreting their products to skin, intestinal, respiratory, or genitourinary tracts.
**Exotoxin** Toxic protein liberated from a bacterial cell.

**Facultative** When describing bacteria without a qualification means ability to grow aerobically or anaerobically.
**Fallopian tubes** Tubes extending from ovaries to uterus.
**Fascia** Sheets of specialized connective tissue.
**Fauces** Area between the mouth and the pharynx. Bounded by the tonsils, soft palate, and base of tongue.
**Febrile** Having a raised temperature.
**Felinophobe** Cat hater.
**Fibrin** Insoluble protein of blood clots.
**Fibrinogen** Precursor of fibrin.
**Fibroblast** Specialized cell producing collagen and elastic connective tissue.
**Fibronectin** A glycoprotein widely distributed in connective tissue and coating cells at mucosal surfaces.
**Fibrosis** Formation of collagenous connective tissue.
**Fimbriae** Very fine fibrils on the surface of a bacterium analogous to the larger pili. Often referred to as pili.
**Fistula** An abnormal passage from a hollow organ (eg, intestine).
**Flaccid** Loose; absence of muscle tone.
**Flagellum** Organelle of motion of bacteria and some eukaryotic cells.
**Fluke** Flat parasitic worm (trematode).
**Fluorochrome** A fluorescent dye.
**Follicle** A small sac or cavity.
**Folliculitis** Usually describes localized inflammation of hair follicles without the purulence of furuncles.
**Fomites** Inanimate objects transmitting infectious agents.
**Foramina** Outlets to cavities.
**Fulminant** Rapid and severe development (eg, of an infection).
**Fungemia** Fungi in the bloodstream.
**Funiculitis** Inflammation a cord-like structure, usually the spermatic cord.
**Furuncle** Purulent infection of a hair follicle; a boil.
**Fusiform** Tapering at both ends.

**Gametocyte** Male or female sexual cell of the malarial parasite found in the blood of humans and transmissible to mosquitoes.
**Ganglion** Group of nerve cells outside the spinal cord.
**Gangrene** Death of tissue.
**Gastro-** pertaining to the stomach.
**-genic** arising from, origin.
**Genital primordium** First recognizable embryonic genital structure. Assists in distinguishing hookworm from *Strongyloides* larvae.
**Genome** The total gene complement of an organism.
**Genotype** The genetic constitution of an organism.
**Geophagia** Eating soil.
**Giemsa stain** A combination of basic and acidic dyes used to stain blood smears and to demonstrate some protozoa.
**Gingival crevice** Area between the tooth and the gums.
**Gingivo-** pertaining to the gums.
**Glaucoma** Excessive pressure in eyeball that can lead to blindness.
**Glia** Supporting cells of the central nervous system (neuroglia).
**Glomerulus** Microscopic organ of specialized capillaries in the kidney that filters waste products from the blood.
**Glottis** The sound-producing area of the larynx.
**Glucans** Polymers of glucose.
**Gnotobiotic animals** Animals reared under aseptic conditions which may either be sterile ("germ free") or in which defined microflora are introduced.
**Gonads** Ovaries or testes.
**Granulocyte** Polymorphonuclear leukocyte of the neutrophil, basophil, or eosinophil series.
**Granuloma** Chronic inflammatory lesion infiltrated with macrophages and lymphocytes and accompanied by fibroblast activity.
**Gravid** Pregnant.
**Guillain-Barré syndrome** Febrile polyneuritis with muscle weakness; may lead to paralysis.
**Gumma** Tertiary syphilitic granulomatous lesion, usually without demonstrable pirochetes.

**Halophilic** Preferring or requiring a high salt content (eg, for growth).
**Haploid** Half the number of chromosomes of eukaryotic tissue cells (see Meiosis) or number of chromosomes in asexual organisms.
**Hapten** A small molecule that can react with a specific antibody but does not elicit antibody production unless attached to a larger molecule.

**Helminth** A parasitic worm.
**Hemagglutination** Agglutination of erythrocytes.
**Hematocrit** Volume of erythrocytes in blood as a percentage of the total volume of blood (adult normal = 45%).
**Hematogenous** Derived from blood. Spread by the bloodstream.
**Hematoma** Extravasation of blood into the tissues causing a swelling.
**Hematopoietic system** Precursor cells that produce blood cells.
**Hematoxylin–eosin stain** Commonly used histological stain. Hematoxylin stains nuclei blue. Eosin is a red counter stain.
**Hematuria** Blood in the urine.
**Hemianopsia** Loss of vision in half the visual field.
**Hemo-, Hema-** Pertaining to blood.
**Hemoglobulinemia** Free hemoglobin in the blood.
**Hemolysin** A substance or enzyme causing lysis of erythrocytes.
**Hemolysis** Liberation of hemoglobin from red cells.
**Hemolytic–uremic syndrome** A syndrome that includes hemolytic anemia, thrombocytopenia, and evidence of renal disease.
**Hemoptysis** Coughing up of blood.
**Hemothorax** Blood in the pleural cavity of the chest.
**Hepato-** Pertaining to the liver.
**Hepatocellular** Pertaining to liver cells (hepatocytes).
**Hepatocytes** Liver cells.
**Hepatoma** Malignant tumor of liver cells.
**Hetero-** Of different origin.
**Heterologous** Derived from a different clone, strain, species or tissue.
**Heterophil antibody** Antibody reacting with an antigen other than that which elicited its production.
**Heteroploid** Eukaryotic cell with abnormal number of chromosomes.
**Heterotroph** An organism that requires organic carbon for nutrition.
**Heterozygous** Possessing different alleles at a particular genetic locus in a diploid cell.
**Hexacanth** A tapeworm embryo containing six pairs of hooklets.
**Hexamer** In virology, a capsomer comprising six subunits.
**Hilar lymph nodes** Nodes at the root of the lung.
**Histiocyte** Tissue macrophage.
**Histocompatibility** Antigens on tissue cells that are recognized by the host as self or foreign.
**HIV-1 or -2** Abbreviation for human immunodeficiency viruses, the cause of AIDS.
**Hodgkin's disease** A malignant lymphoma initially affecting groups of lymph nodes.
**Homeostasis** Tendency to stability of conditions within a complex biological system.
**Homonymous hemianopsia** Blindness affecting the same half of the visual field in each eye.
**Homozygous** Possessing the same alleles at a particular genetic locus in a diploid cell.
**Humoral** Mediated by fluids. In immunology relates to antibody mediated immunity as opposed to cellular immunity.
**Hyaline** Clear and transparent.
**Hyaluronic acid** Acid mucopolysaccharide comprising the ground substance of connective tissue. Also found in synovial fluids.
**Hybridization** Process in which denatured, single stranded nucleic acids from different sources are annealed. Homologous sequences form double strands that can be detected and quantified.
**Hybridoma** A clone derived from fused cells of different origin (eg, from an antibody producing lymphocyte and a tumor cell).
**Hydrocele** Fluid accumulation within the scrotum.
**Hydrocephalus** Pathological accumulation of cerebrospinal fluid in the ventricles of brain.
**Hydronephrosis** Accumulation of urine in the renal pelvis due to obstruction of urinary flow. Associated with atrophy of the renal parenchyma.
**Hyper-** Greater than, above normal.
**Hyperalimentation** Intravenous administration of nutrients for treatment of actual or potential malnutrition.
**Hyperammonemia** Excessive amounts of ammonia in the blood.
**Hyperbaric oxygen** Oxygen under increased pressure relative to the atmosphere.
**Hyperemia** Increased blood flow to a tissue.
**Hypernatremia** Increased serum sodium.
**Hyperplasia** Increase in the number of cells in a tissue.
**Hypersensitivity** Exaggerated and harmful immune response to a normally innocuous antigenic stimulus.
**Hypertension** Elevated blood pressure.
**Hypertonic** Of higher osmotic pressure than fluid on the other side of a semipermeable membrane (eg, cell membrane).
**Hypertrophy** Enlargement of an organ due to increase in size of its cells. Note distinction from hyperplasia.
**Hypha** A fungal filament.
**Hypo-** Less than, below normal.
**Hypochlorhydria** Reduced hydrochloric acid in the stomach.
**Hypoglycemia** Blood sugar below normal levels.
**Hypotension** Low blood pressure.
**Hypothalamus** Portion of the brain that forms the floor and part of the lateral wall of the third ventricle.
**Hypothermia** Serious reduction in body temperature.
**Hypoxia** Decreased oxygen supply to the tissues.

**Icosahedron** A solid geometric shape having 12 vertices. Serves as the structural basis for many viruses.
**Icteric** Pertaining to jaundice.
**Idiopathic** Of unknown origin.
**Ig** Abbreviation for immunoglobulin antibodies. Classes include IgG, IgM, IgA, IgD, IgE, and sIgA.

**Ileitis** Inflammation of the lower ileum.
**Ileum** Portion of the small intestine between the jejunum and the cecum.
**Immunocompromise** Deficiency in some components of the body's immune mechanisms.
**Immunocyte** Cell of the lymphoid series that responds to an antigenic stimulus by producing antibodies or initiating cell mediated immune processes.
**Immunodiffusion** A procedure involving diffusion of antigen and antibody towards each other in a gel. A visible precipitate develops where optimal concentrations interact.
**Immunofluorescence** A serologic procedure using antibody labeled with a fluorescent dye that allows visible detection of sites of reaction with antigen.
**Immunogen** An antigen that induces an immune response.
**Immunoglobulins** Large class of glycoproteins that constitute the antibodies produced in response to antigenic stimuli.
**Impetigo** Superficial pustular skin infection.
**In vitro** Occurring in the test tube.
**In vivo** Occurring in the living animal.
**Inclusion body** A morphologically distinct intracellular mass of viruses or virus components.
**Infarct** Interference with the blood supply producing local death of tissue.
**Integument** Skin.
**Integrins** Family of transmembrane proteins of eukaryotic cells that interact with extracellular matrix and cytoskeleton proteins
**Inter-** Between.
**Interferon** Class of cytokine proteins. When produced by virally infected cells they inhibit viral replication in these and adjacent cells.
**Interleukin** Class of cytokine produced by macrophages or T cells that mediate immune responses.
**Interstitial** Spaces between the cells of a tissue.
**Intertriginous** Pertaining to area between folds of the skin.
**Intima** Inner lining of a blood vessel.
**Intra-** Within.
**Intrapartum** Occurring during the process of childbirth.
**Intrathecal** Within the membranes of the spinal cord.
**Introitus** An opening.
**Isoantigen** Normal substance present in one individual that may elicit an antibody response in another.
**Isotonic** Of the same osmotic pressure as a solution on the other side of a semipermeable membrane.
**-itis** inflammation.

**Janeway's lesions** Painless macular lesions of palms and soles seen in acute bacterial endocarditis.
**Jejunum** Portion of small intestine between duodenum and ileum.

**Kaposi's sarcoma** Multiple malignant vascular tumors. Occur most commonly as a complication of AIDS.
**Karotype** Size, structure, and organization of chromosomes within a cell.
**Karyosome** Area of chromatin concentration in a cell nucleus.
**Keratin** Major protein of the skin, hair, and nails.
**Keratitis** Inflammation of the cornea of the eye.
**Kilobase** Unit to describe the lengths of a nucleotide sequence. One kilobase = 1000 nucleotides.
**Kinetoplast** Structure at the base of a protozoal flagellum.
**Kupffer cells** Fixed phagocytic cells of the liver sinusoids. Part of the reticulo-endothelial system.
**Kwashiorkor** Condition caused by severe protein malnutrition in children.

**Labia** Structures of the external female genitalia.
**Lactoferrin** Iron-binding protein present in milk, other secretions, and granules of neutrophil leukocytes.
**Lamina propria** Connective tissue supporting the epithelial cells of a mucous membrane.
**Laminin** Major protein component of basal lamina.
**Latex beads** Used to adsorb soluble antigens. The treated beads agglutinate with specific antibody.
**Leukemia** Malignant tumor of white blood cells.
**Leuko-** White; relating to a leukocyte.
**Leukocyte** White blood cells including granulocytes, lymphocytes, and monocytes.
**Leukocytosis** Increased blood leukocyte count.
**Leukopenia** Abnormally low leukocyte count.
**Leukotrienes** Products of arachidonic acid that mediate inflammatory and allergic reactions.
**Ligand** One component of a complex involving the binding of molecules or structures.
**Lipo-** Relating to fats or lipids.
**Lobar** Related to a lobe of the lung.
**Lophotrichous** Describing several flagella at one or both ends of a bacillus.
**Lumen** Cavity within a tubular organ.
**Lupus erythematosus (systemic)** Autoimmune inflammatory disease of skin, joints, and other tissues.
**Lymph** Tissue fluid derived from the bloodstream and passing to the lymphatics.
**Lymphadenitis** Enlarged, inflamed lymph nodes.
**Lymphangitis** Inflammation of lymphatic vessels.
**Lympho-** Pertaining to the lymphatic system.
**Lymphocytosis** Increased blood lymphocyte count.
**Lymphokine** Cytokine produced by lymphocytes.
**Lymphoma** Tumor of lymphatic tissues.
**Lymphoreticular** Relating to the reticuloendothelial system.
**Lysis** Dissolution of cells.
**Lysosome** Intracellular granules of cells that contain hydrolytic digestive enzymes.
**Lysozyme** Enzyme that breaks down peptidoglycan.
**-lytic** Pertaining to lysis.

**Macro-** Large.
**Macrocytic anemia** Anemia characterized by large erythrocytes.

**Macrophage** Tissue phagocyte derived from blood mononuclear cells.
**Macule** A flat lesion of skin rash.
**Masseter** Major muscle controlling movement of the lower jaw.
**Mast cell** Connective tissue cell analogous to the blood basophil. Granules contain heparin, histamine, and other vasoactive mediators.
**Mastitis** Inflammation of the breast.
**Mastoid** Process of temporal bone behind the ear that contains air cells.
**Matrix** Extracellular substance of tissues.
**Meatus** Orifice.
**Meckel's diverticulum** Congenital diverticulum of the lower part of the ileum.
**Mediastinum** Mid-portion of the chest including heart, bronchial bifurcation, and esophagus.
**Medulla** The inner portion of an organ within the cortex.
**Medulla oblongata** Portion of central nervous system between the brain and spinal cord.
**Mega-** Large.
**Megacolon** Dilatation of the colon.
**-megaly** Enlargement, usually of an organ.
**Meiosis** Cellular division process yielding haploid gametes.
**Meninges** The membranes covering the brain and the spinal cord.
**Meningomyelocele** Malformation of vertebral column with protrusion of meninges.
**Mentation** Mental activity; thinking.
**Merozoite** A stage in the life cycle of a sporozoan parasite resulting from asexual division; a daughter cell.
**Mesenchymal** Derived from the embryonic mesoderm layer.
**Mesentery** Fold of peritoneum surrounding the intestinal tract and attaching it to the posterior abdominal wall.
**Mesophile** A microbe that grows best at temperatures of approximately those of the body.
**Mesosome** A complex invagination of the bacterial cell membrane.
**Metastases** Satellite tumors or infections spread through lymphatics or the bloodstream from a primary site.
**-metry** measure.
**Micro-** Small.
**Microaerophilic** Can grow only in less than the atmospheric concentration of oxygen, or anaerobically.
**Microcephaly** Small head with failure of development of the brain.
**Microphthalmia** Failure to develop normal sized eyes.
**Microtubule** Cylindrical cytoskeletal element of animal and plant cells.
**Mitochondria** Complex cytoplasmic organelles of eukaryotic cells involved in oxidative phosphorylation.
**Mitogen** Substance that increases the normal frequency of mutations.
**Mitral valve** Valve between the left atrium and ventricle of the heart.
**Monoclonal** Derived from a single cell.
**Monocyte** Large mononuclear phagocyte of the blood. Precursor of the macrophage.
**Monolayer** A single layer of cultured eukaryotic cells on a glass or plastic surface.
**Monotrichous** Possessing a single flagellum.
**Mordant** Substance that enhances the effect of a stain.
**Morphology** The shape, size, and form of an organism or cell.
**Mucolytic** Substance that dissolves mucus.
**Multiple sclerosis** Chronic disorder involving disseminated focal damage to nerve cells.
**Mutagen** Substance that increases the mutation rate of cells or organisms.
**Myalgia** Pain in the muscles.
**Mycelium** A mass of fungal hyphae.
**Mycetoma** A localized granuloma or lesion caused by a fungus.
**Mycosis** A fungal infection.
**Myelin** Component of the myelin sheath around the axon of a neuron that increases the conduction velocity of the nerve impulse.
**Myelitis** Inflammation of the spinal cord.
**Myeloma** Malignant tumor derived from bone marrow cells.
**Myeloperoxidase** Intracellular enzyme of professional phagocytes.
**Myo-** Pertaining to muscle.
**Myocardium** Heart muscle.
**Myringitis** Inflammation of the tympanic membrane of the ear.

**Nares** Interior of the nostrils.
**Nasal turbinates** Three scroll-like bony projections from the lateral wall of the nasal cavity (nasal conchae).
**Nasolacrimal duct** Duct draining the conjunctiva into the nasal cavity.
**Necrosis** Death of tissue.
**Neo-** New.
**Neoplasm** Tumor.
**Nephrito-** Pertaining to the kidney.
**Nephritogenic** Producing inflammation of the kidneys.
**Neuro-** Pertaining to the central nervous system or nerves.
**Neuromotor synapses** Connections between nerve endings and muscle.
**Neurone** Nerve and its nerve cell.
**Neutropenia** Reduced number of circulating neutrophil leukocytes.
**Neutrophils** Major class of polymorphonuclear phagocytic leukocytes.
**NGU** Nongonococcal urethritis.
**Nidus** Focus of infection, a cluster.
**Noma** A gangrenous condition spreading from the oral cavity to the skin; seen in undernourished children.
**Nosocomial** Acquired within a hospital.
**Nucleocapsid** The nucleic acid-protein complex found inside an enveloped virus.

**Nucleoid** The double stranded circular DNA genome of a bacterium.
**Nucleolus** Round body within a eukaryotic nucleus that is the site of synthesis of ribosomal RNA.

**Occult** Hidden, inapparent.
**Olfactory** Pertaining to the sense of smell.
**Olfactory bulb** Terminal enlarged portion of the olfactory tract from which the olfactory nerves emerge.
**Oligo-** Small, few.
**Oligodendroglia** Specialized connective tissue of the central nervous system.
**Onco-** Pertaining to tumors.
**Oncogene** Gene whose activation is associated with malignant change and progression.
**Ontogeny** Origin and course of development of an individual organism.
**Operculum** A lid or cover.
**Operon** Operator gene and the adjacent structural gene(s) that it controls.
**Ophthalmia** Severe inflammation of the eye.
**Opisthotonos** Severe spasm of back muscles leading to hyperextension of the spine.
**Opportunist** A microorganism that only causes disease when the body's defenses are compromised or bypassed.
**Opsonin** Antibody or complement component that facilitates phagocytosis when bound to a microorganism.
**Orbit** Skull cavity that contains the eyeball.
**Orchitis** Inflammation of a testis.
**Organelles** Membrane-bound cytoplasmic structures of eukaryotic cells (eg, mitochondria).
**Organogenesis** Formation of the organs of the body.
**Oro-** Pertaining to the mouth.
**-oscopy** Use of an instrument to see within a viscus or vessel.
**Osler's nodes** Skin papules, usually of hands and feet, seen in bacterial endocarditis.
**Ossicles** Small bones (eg, of hearing).
**Osteo-** Pertaining to bone.
**Osteomyelitis** Inflammation of bone marrow and adjacent bone.
**Oto-** Pertaining to the ear.
**Oviparous** Producing eggs from which the embryo is released outside the body.
**Oxidase** Oxidation-reduction enzyme that catalyzes transfer of electrons to molecular oxygen with formation of water.

**Pan-** All, throughout.
**Pandemic** Worldwide severe epidemic.
**Panencephalitis** Inflammation of all tissues of the brain.
**Papilla** Small nipple-like swelling.
**Papilledema** Edema of the optic nerve and adjacent retina.
**Papilloma** Warty tumor of the epithelium.
**Papule** Small, firm, elevated nodule on the skin.
**Para-** Beside, abnormal.
**Parasite** An organism that lives on and at the expense of another organism.
**Parasitism** Describes the relationship between parasite and host.
**Parenchymal** Substance of body organs in contrast to their covering.
**Parenteral** Administration by injection rather than by mouth.
**Paresis** Paralysis.
**Paresthesias** Disorders of sensation; tingling.
**Paronychia** Infection of nail fold.
**Parotid glands** Salivary glands beneath the cheek.
**Parturition** The process of giving birth.
**Pathogenic** Capable of causing disease.
**Pathognomonic** Diagnostic, distinctive.
**-pathy** denoting disease.
**-penia** decreased numbers.
**Pentamer** A polymer of viral capsid having five structural units.
**Peptidoglycan** High molecular weight cross-linked polymer forming the rigid structure of the bacterial cell wall.
**Peptone** Protein hydrolysed product used as a source of amino acids in bacterial culture media.
**Peri-** Around, covering.
**Periapical** Beside the root of a tooth.
**Pericardium** Membranous lining around the heart.
**Perineum** Area between vulva or scrotum and the anus.
**Periodontal** Area around the tooth including supporting tissues.
**Perioplasm** Area between the outer and cell membranes of a Gram-negative bacterium. Contains the peptidoglycan layer.
**Periosteum** Membrane around the bone.
**Peristalsis** Normal contractile waves of a hollow organ.
**Peristome** The mouth and surrounding areas of certain ciliated protozoa.
**Peritrichous** Presence of multiple flagella around a bacterial cell.
**Permease** A protein of the bacterial cell membrane transport system.
**Petechiae** Small ($<3$ mm) hemorrhages in the skin containing red blood cells or hemoglobin.
**Peyer's patches** Lymphoid follicles in the ileum.
**Phage** Common abbreviation for bacteriophage.
**Phagocyte** A cell that ingests foreign material.
**Phagolysosome** The digestive vacuole formed by fusion of the cell lysosomes with the phagocytic vacuole.
**Phenotype** The properties expressed by the complete genome under particular conditions.
**Pheromone** Hormone-like substance that elicits a favorable or attraction response in an individual of the same species.
**-phobia** fear of, repulsion.
**Phonation** Speech.
**Photophobia** Intolerance of light.
**-phylia** affection for.
**Phylogeny** Pertaining to the evolution of a species.

**PID** Pelvic inflammatory disease.
**Pilo-sebaceous** Unit of hair follicle and sebaceous gland.
**Pilus** Fibrillar structure on the surface of a bacterial cell.
**Pinocytosis** Uptake of fluids into a cell by a mechanism analogous to phagocytosis.
**Plankton** Minute free-floating organisms, vegetable and animal, which live in natural waters.
**Plaque** A patch or flat area. An area of lysis in fixed host cells by an infecting virus.
**Plasma** Noncellular component of whole blood.
**Plasmid** Extrachromosomal circular double stranded DNA molecule.
**Plasmin** Derived from plasminogen–dissolves fibrin.
**Platelet** Small anucleate cell involved in filling small holes in blood vessels and in clotting mechanisms.
**Pleo-** More.
**Pleocytosis** Increased number of cells in a particular area.
**Pleomorphism** Variation in shape and size.
**Pleura** Membrane covering the lungs and thoracic cavity enclosing the pleural space.
**Pleurisy** Inflammation of the pleura.
**Pleuro-** Relating to the pleura.
**Pleurodynia** Pain caused by inflammation or irritation of the pleura.
**Pneumocyte, Type I** Flat cell lining alveoli of lung which is involved in gas exchange.
**Pneumocyte, Type II** Rounded surfactant-producing cell in alveoli of the lung.
**Pneumonitis** Inflammation of the lung.
**Pneumothorax** Air in the pleural cavity.
**Poly-** Many, repeated.
**Polyarthralgia** Pain in several joints.
**Polycistronic** Encoding two or more proteins (eg, polycistronic mRNA).
**Polyclonal activation** Simultaneous activation of different antibody producing clones of lymphocytes.
**Polymerase chain reaction** Continuous enzyme-mediated amplification of a nucleotide sequence that allows its detection and analysis.
**Polymorphonuclear** Two or more lobes to the nucleus.
**Polymyositis** Inflammation of many muscles.
**Polyneuritis** Inflammation of many nerves.
**Polyp** A sessile benign or malignant tumor of a mucous membrane (usually of colon).
**Polyposis** Presence of many polyps.
**Porin** Protein of outer membrane pores of Gram-negative bacteria.
**Portal venous system** Veins carrying blood from the intestinal tract to the liver.
**Premenarcheal** Prepubertal years in the female (before onset of menses).
**Prepuce** Foreskin.
**Pro-** Before, a precursor.
**Proctoscopy** Use of an instrument to examine interior of the rectum.
**Prodromal** Initial symptoms before the characteristic manifestations of disease develop.
**Proglottid** One of the segments of the body of a tapeworm.
**Prokaryote** Organism lacking a true nucleus. Possesses a single chromosome.
**Prophage** Complete bacterial virus genome integrated in the chromosome.
**Prophylaxis** Measures or treatments designed to prevent disease.
**Prostaglandins** Derivatives of arachidonic acid that mediate a variety of biological reactions including inflammation.
**Prostate gland** Gland surrounding the male urethra that produces part of the seminal fluid.
**Prosthesis** Artificial replacement of a missing part of the body.
**Proteinuria** Protein in the urine indicating a renal abnormality.
**Prothrombin** Precursor of thrombin; thrombin activates the terminal blood clotting mechanism.
**Protomer** Protein subunit of a viral capsomere.
**Protoplasm** The viscid colloidal solution that makes up living matter.
**Protoplast** A Gram-positive bacterium that has lost its cell wall.
**Prototroph** Bacterial strains with complete synthetic pathways from which auxotrophs may be derived.
**Protozoan** A unicellular member of the animal kingdom.
**Proventriculus** An enlargement of the alimentary tract of an invertebrate that precedes the stomach.
**Provirus** Complete viral genome integrated into a eukaryotic genome.
**Pruritus** Itching.
**Pseudo-** False.
**Pseudopod** A pseudopodium. Moving extrusion of the cytoplasm of an amoeboid cell that brings about movement or ingestion of food particles.
**Psychrophile** A microorganism that grows best or exclusively at low temperatures.
**Puerperal** Following childbirth.
**Purpura** Multiple hemorrhages in the skin, mucous membrane, or other organs.
**Pustule** Pus in an infected hair follicle or sweat gland producing a visible inflammatory swelling.
**Pyelonephritis** Infection of the pelvis and tissues of the kidney.
**Pylephlebitis** Inflammation in the portal venous system.
**Pyo-** Producing pus.
**Pyogenic** Producing pus and pustular lesions.
**Pyuria** Pus in the urine.

**Radioimmunoassay** A method for detecting antigen-antibody reactions that uses a radioisotope as a readily detectable label.
**Rales** Crackling respiratory sounds heard with the stethoscope.
**Receptor** Component of the cell surface to which another substance or organism attaches specifically.

**Redox potential** Oxidation–reduction potential.
**Reduviid** A large winged "cone-nosed" insect.
**Renal** Pertaining to the kidney.
**Repressor** A regulatory protein that binds to an operator sequence and inhibits expression of the adjacent gene.
**Reservoir of infection** Natural habitat or source of an infecting organism.
**Reticuloendothelial system** System of phagocytic monocytes, particularly those in the spleen, bone marrow, and lymph nodes.
**Retinoblastoma** Malignant tumor of the retina.
**Retrovirus** RNA virus, the genome of which is transcribed into DNA by its reverse transcriptase.
**Reverse transcriptase** RNA-directed DNA polymerase.
**Rhino-** Pertaining to the nose.
**Rhinorrhea** Continuous discharge of watery mucus from the nose.
**Rhonchi** Coarse snoring or rattling respiratory sounds heard with a stethoscope.
**RIA** *See* Radioimmunoassay.
**Romana's sign** Unilateral ophthalmia, edema of the eyelids, and enlarged draining lymph nodes.
**Rostellum** Portion of tapeworm head that contains hooklets or other attachment organs.

**Salpingitis** Inflammation of the fallopian tubes.
**Saprophyte** Organism living on dead organic material in the environment.
**Sarcoidosis** Disease of unknown etiology characterized by granulomatous lesions of many tissues and organs.
**Sarcolemma** Membrane surrounding muscle fibers.
**Schizogony** Asexual reproduction in sporozoa producing merozoites by multiple nuclear fusion followed by cytoplasmic segregation.
**Schizont** The multinucleated stage of a sporozoan undergoing schizogony.
**Sclera** White part of the eyeball.
**Scolex** The attachment organ or head of a tapeworm.
**-scopy** Denotes use of an instrument for visual examination of a hollow viscus (eg., bronchoscopy).
**Scotoma** A blind spot in the visual field.
**Sebaceous** Relating to sebum and sebum production.
**Sebum** Waxy secretion of sebaceous glands.
**Seminal vesicles** Sacs in which semen is stored prior to ejaculation.
**Sepsis** A term often used synonymously with septicemia, but implies the presence of circulating infectious agents.
**Septicemia** Evidence of systemic disease associated with presence of organisms in the blood (*see* Bacteremia).
**Sequelae** Results occurring subsequent to an infection or other disease.
**Sequestrum** Necrotic bony fragment.
**Seroconversion** Development of antibodies in response to an infection.
**Serodiagnosis** Diagnosis of an infection by serologic procedures.
**Serotonin** Vasoconstricting amine usually derived from platelets
**Serotype** Subtype of species detectable with specific antisera.
**Serpiginous** Moving irregularly from one place to another, snake-like.
**Serum** Liquid part of blood separable after clotting.
**Shunt** Deviation of blood or other body fluids (eg, from artery to vein).
**Sickle cell anemia** Hereditary anemia associated with crescent-shaped erythrocytes resulting from an abnormal hemoglobin.
**Siderophore** Compound that binds iron.
**Sigmoid colon** Lower portion of the colon between descending colon and rectum.
**Sinus** A tract leading from an infected area or hollow viscus to the surface; a wide venous blood channel; accessory nasal sinuses that are blind sacs draining to the nasopharynx.
**Sinusoid** A wide thin-walled venous passage. Smaller than a sinus.
**Slime layer** Term sometimes used for polysaccharide surface components of bacteria that do not constitute a morphologic capsule.
**Spasticity** Excessive tone of muscles leading to awkward movement.
**Spheroplast** A circular, osmotically unstable, Gram-negative rod that has lost its peptidoglycan layer.
**Sphincter** Circular muscle controlling a natural orifice.
**Splanchnic** Pertaining to the viscera.
**Spleno-** Relating to the spleen.
**Sporogony** Sexual reproduction process in sporozoan parasites leading to formation of oocysts and sporozoites.
**Sporozoite** Motile, elongated, infective stage of sporogony.
**Sprue** A chronic form of intestinal malabsorption.
**Squamous epithelium** Composed of layers of flattened cells.
**Stasis** Stagnation or cessation of flow of body fluids.
**Stenosis** Reduction in diameter of a blood vessel or tubular organ.
**Steroids** Derivatives of cholesterol including hormones, some of which have anti-inflammatory effects.
**Sterol** Lipid-soluble steroid with long aliphatic side chains. Present in eukaryotic cell membranes as cholesterol or ergosterol.
**Stevens-Johnson syndrome** A serious allergic reaction, characterized by multiple blister-like lesions of skin and mucous membrane.
**Stomatitis** Inflammation of the mouth.
**Strabismus** Squint.
**Stratum corneum** Outer keratinized part of the skin.
**Stridor** Harsh respiratory sound due to partial respiratory obstruction.
**Strobila** Chain of segments making up the body of a tapeworm.
**Sub-** Below.

**Subarachnoid** Cerebrospinal fluid containing area between the middle (arachnoid) and inner (pia mater) layers of the meninges.
**Subdural** Between the outer (dura mater) and middle (arachnoid) layers of the meninges.
**Submandibular** Below the jaw.
**Subphrenic** Below the diaphragm.
**Sulcus** Groove.
**Suppurative** Producing pus.
**Supra-** Above.
**Surfactant** A substance that acts on a surface to reduce surface tension (eg, a detergent).
**Sylvatic** Pertaining to the woods. Commonly applied to nonurban plague whether occurring in wooded or prairie land.
**Symbiont** An organism living on or in close association with another.
**Synapse** A connection between neurons for nerve impulse transmission.
**Syncytium** A multinucleate mass of fused cells.
**Syndrome** Group of clinical manifestations characterizing a particular disease or condition.
**Synergistic** Enhanced rather than additive effect of two agents or processes acting together.
**Synovium** Lining membrane of a joint, tendon, or bursa.

**T cells** Thymus derived immunocytes: helper, suppressor, and cytotoxic T cells.
**Tachy-** Increased rate, swift.
**Tachypnea** Abnormally rapid rate of breathing.
**Talin** One of the proteins that connects integrins to the actin cytoskeleton of eukaryotic cells.
**Tamponade (cardiac)** Increased fluid or constriction around the heart leading to interference in cardiac function.
**Tenesmus** Ineffective and painful straining at stool or urination.
**Tenosynovitis** Inflammation of a tendon sheath.
**Teratogenic** Causing abnormalities of fetal development.
**Thalassemia** Hereditary hemolytic anemia resulting from abnormal hemoglobin synthesis.
**Thermo-** Pertaining to heat.
**Thermophile** Bacteria with an optimal growth temperature of over 50°C.
**Thrombo-** Pertaining to thrombosis.
**Thrombocyte** See platelet.
**Thrombocytopenia** Abnormally low platelet count.
**Thrombophlebitis** Inflammation of a vein with thrombosis; may release infected emboli.
**Thrombus** A blood clot developing in vivo.
**Thymus** A lymphoid organ located in the anterior upper portion of the mediastinum. The site of maturation of T cells.
**Titer** Highest dilution of an active substance (eg, antibody in serum) that still causes a discernible reaction (eg, an agglutination reaction).
**Tracheo-** Pertaining to the trachea.
**Tracheostomy** Surgically produced artificial air passage to the trachea.
**Trans-** Across.
**Transcriptase** DNA-directed RNA polymerase.
**Transferrin** Serum protein that binds and transports iron.
**Transovarial** Passage of infectious agents to progeny by way of the egg. Usually occurs in ticks and mites.
**Transposon** A DNA segment carrying one or more recognizable genes that can move between plasmid and between plasmid and chromosome in both directions.
**Trimester** Usually means a three-month period of pregnancy.
**Trismus** Spasm of the masseter muscle; lockjaw.
**Trophozoite** The motile feeding stage of a protozoan parasite.
**Tropism** Having an affinity for a particular organ, or moving towards or away from a particular stimulus.
**Tubulin** Protein subunit of microtubules.
**Tumorigenesis** The property of causing tumors.
**Turgor pressure** Osmotic pressure of the cellular contents.
**Tympanic membrane** Eardrum.

**Ultrasonograph** Picture of deep organs of the body derived from reflection of ultrasonic waves.
**Uremia** Toxic accumulation of nitrogenous metabolites due to renal insufficiency.
**Ureter** Tube carrying urine from the kidney to bladder.
**Urethra** Tube carrying urine from the bladder to the exterior.
**-uria** Pertaining to urine.
**Uropathic** Causing disease of the urinary tract.
**Urticaria** Local edema and itching of the skin.
**Uvea** Inner vascular coat of the eyeball, including the iris.
**Uvula** Small extension hanging from the back of the soft palate.

**Vacuolate** Forming small holes or vacuoles.
**Vacuole** Microscopic hole or cavity.
**Vagotomy** Surgical cutting of the vagus nerve.
**Vasa vasorum** Small blood vessels in walls of veins and arteries.
**Vasculitis** Inflammation of blood vessels.
**Vaso-** Pertaining to blood vessels.
**Vector** An aminate transmitter of disease (eg, an insect).
**Venipuncture** Insertion of a hypodermic needle into a vein—usually to draw blood.
**Ventricle** Fluid cavity (eg, chamber of the heart).
**Vesicle** Small fluid filled cavity (eg, a blister-like lesion of the skin).
**Vesicoureteral junction** Junction of ureter with the urinary bladder.
**Vestibular function** Function of the vestibular branch of the eighth cranial nerve concerned with the body's equilibrium.
**Vinculin** One of the proteins that connects integrins to the actin cytoskeleton of eukaryotic cells.

**Viremia** Presence of a virus in the bloodstream.
**Virion** A complete virus particle.
**Viropexis** Viral entry into the cell by phagocytosis.
**Viruria** Viruses in the urine.
**Viscera** Interior organs of the body (eg, the intestinal tract).
**Vitreous humor** The clear viscous fluid in the posterior chamber of the eye.
**Vitronectin** Protein component of extracellular matrix.
**Viviparous** Developing young within the body as opposed to oviparous.

**Western blot** Test for antibodies to specific proteins separated by gel electrophoresis.
**Whitlow** Abscess of the terminal pulp of the finger. Also paronychia.
**Wright's stain** Stain for blood cells that has similar properties to Giemsa stain.

**Xenodiagnosis** Recovery of a parasite by allowing an arthropod to feed on the patient and seeking the parasite in the arthropod.
**Xerostomia** Dry mouth from dysfunction of the salivary glands.

**Zoonosis** A disease transmittable to humans from an animal host or reservoir.
**Zygote** The cell that results from fusion of male and female gamete.
**Zymodeme** An isoenzyme typing pattern.

# INDEX

# INDEX

Page numbers followed by *t* and *f* indicate tables and figures, respectively. Page numbers in **boldface** indicate major discussions.

## B

## C

## E

## F

## G

## H

## M

## O

## P

## Q



## R

## S

## T

## U



## V