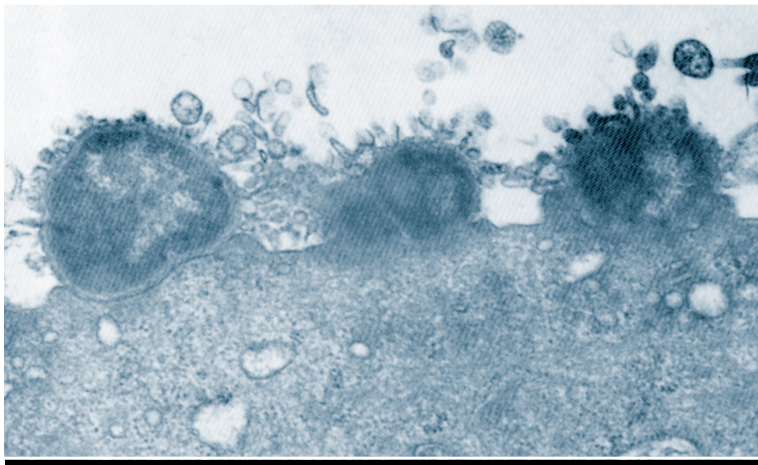
chapter 17

Immune Response to Infectious Diseases



Neisseria gonorrheae Attaching to Urethral Epithelial Cells

If a pathogen is to establish an infection in a susceptible host, a series of coordinated events must circumvent both innate and adaptive immunity. One of the first and most important features of host innate immunity is the barrier provided by the epithelial surfaces of the skin and the lining of the gut. The difficulty of penetrating these epithelial barriers ensures that most pathogens never gain productive entry into the host. In addition to providing a physical barrier to infection, the epithelia also produce chemicals that are useful in preventing infection. The secretion of gastric enzymes by specialized epithelial cells lowers the pH of the stomach and upper gastrointestinal tract, and other specialized cells in the gut produce antibacterial peptides.

A major feature of innate immunity is the presence of the normal gut flora, which can competitively inhibit the binding of pathogens to gut epithelial cells. Innate responses can also block the establishment of infection. For example, the cell walls of some gram-positive bacteria contain a peptidoglycan that activates the alternative complement pathway, resulting in the generation of C3b, which opsonizes bacteria and enhances phagocytosis (see Chapter 13). Some bacteria produce endotoxins such as LPS, which stimulate the production of cytokines such as TNF-α, IL-1, and IL-6 by macrophages or endothelial cells. These cytokines can activate macrophages. Phagocytosis of bacteria by macrophages and other phagocytic cells is another highly effective line of innate defense. However, some types of bacteria that commonly grow intracellularly have developed mechanisms that allow them to resist degradation within the phagocyte.

Viruses are well known for the stimulation of innate responses. In particular, many viruses induce the production of interferons, which can inhibit viral replication by inducing an antiviral response. Viruses are also controlled by NK cells. As described in Chapter 14, NK cells frequently form the first line of defense against viral infections.

Generally, pathogens use a variety of strategies to escape destruction by the adaptive immune system. Many pathogens reduce their own antigenicity either by growing within host cells, where they are sequestered from immune attack, or by shedding their membrane antigens. Other pathogens camouflage themselves by mimicking the surfaces of host cells, either by expressing molecules with amino acid sequences similar to those of host cell-membrane molecules or by acquiring a covering of host membrane molecules. Some pathogens are able to suppress the immune response selectively or to regulate it so that a branch of the immune system is activated that is ineffective against the pathogen. Continual variation in surface antigens is another strategy that enables a pathogen to elude the immune system. This antigenic variation may be due to the gradual accumulation of mutations, or it may involve an abrupt change in surface antigens.

Both innate and adaptive immune responses to pathogens provide critical defense, but infectious diseases, which have plagued human populations throughout history, still cause the death of millions each year. Although widespread use of vaccines and drug therapy has drastically reduced mortality from infectious diseases in developed countries, such diseases continue to be the leading cause of death in the Third World. It is estimated that over 1 billion people are infected worldwide, resulting in more than 11 million deaths every year (Figure 17-1). Despite these alarming numbers, estimated expenditures for research on infectious diseases prevalent in the Third World are less than 5% of total health-research expenditures worldwide. Not only is this a tragedy for these countries, but some of these diseases are beginning to emerge or re-emerge in developed countries. For

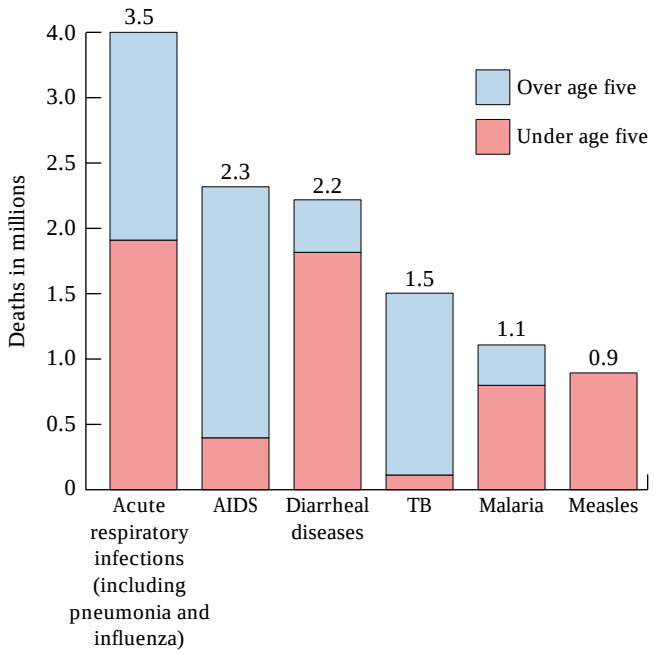


FIGURE 17-1 Leading infectious disease killers. Data collected and compiled by the World Health Organization in 2000 for deaths in 1998. HIV-infected individuals who died of TB are included among AIDS deaths.

example, some United States troops returned from the Persian Gulf with leishmaniasis; cholera cases have recently increased worldwide, with more than 100,000 cases reported in KwaZulu-Natal, South Africa, during the summer of 2001; and a new drug-resistant strain of *Mycobacterium tuberculosis* is spreading at an alarming rate in the United States.

In this chapter, the concepts described in earlier chapters, antigenicity (Chapter 3) and immune effector mechanisms (Chapters 12–16), as well as vaccine development (which will be considered in Chapter 18) are applied to selected infectious diseases caused by viruses, bacteria, protozoa, and helminths—the four main types of pathogens.

Viral Infections

A number of specific immune effector mechanisms, together with nonspecific defense mechanisms, are called into play to eliminate an infecting virus (Table 17-1). At the same time, the virus acts to subvert one or more of these mechanisms to prolong its own survival. The outcome of the infection depends on how effectively the host's defensive mechanisms resist the offensive tactics of the virus.

The innate immune response to viral infection is primarily through the induction of type I interferons (IFN-α and IFN-β) and the activation of NK cells. Double stranded RNA (dsRNA) produced during the viral life cycle can induce the expression of IFN-α and IFN-β by the infected cell. Macrophages, monocytes, and fibroblasts also are capable of synthesizing these cytokines, but the mechanisms that induce the production of type I interferons in these cells are not completely understood. IFN-α and IFN-β can induce an antiviral response or resistance to viral replication by binding to the IFN α/β receptor. Once bound, IFN-α and IFN-β activate the JAK-STAT pathway, which in turn induces the transcription of several genes. One of these genes encodes an

TABLE 17-1 Mechanisms of humoral and cell-mediated immune responses to viruses

Response type	Effector molecule or cell	Activity
Humoral	Antibody (especially, secretory IgA)	Blocks binding of virus to host cells, thus preventing infection or reinfection
	IgG, IgM, and IgA antibody	Blocks fusion of viral envelope with host-cells plasma membrane
	IgG and IgM antibody	Enhances phagocytosis of viral particles (opsonization)
	IgM antibody	Agglutinates viral particles
	Complement activated by IgG or IgM antibody	Mediates opsonization by C3b and lysis of enveloped viral particles by membrane-attack complex
Cell-mediated	IFN-γ secreted by T_H or T_C cells	Has direct antiviral activity
	Cytotoxic T lymphocytes (CTLs)	Kill virus-infected self-cells
	NK cells and macrophages	Kill virus-infected cells by antibody-dependent cell-mediated cytotoxicity (ADCC)

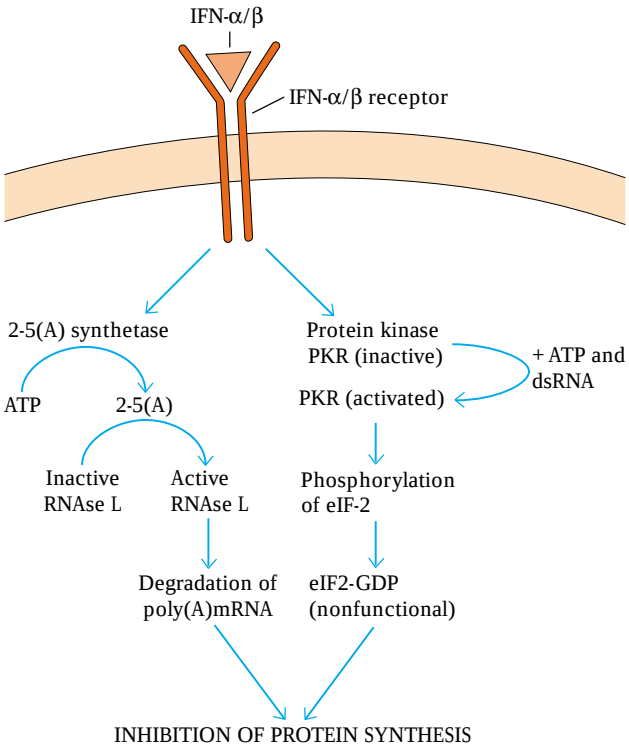


FIGURE 17-2 Induction of antiviral activity by IFN-α and -β. These interferons bind to the IFN receptor, which in turn induces the synthesis of both 2-5(A) synthetase and protein kinase (PKR). The action of of 2-5(A) synthetase results in the activation of RNAse L, which can degrade mRNA. PKR inactivates the translation initiation factor eIF-2 by phosphorylating it. Both pathways thus result in the inhibition of protein synthesis and thereby effectively block viral replication.

enzyme known as 2′-5′-oligo-adenylate synthetase [2-5(A) synthetase], which activates a ribonuclease (RNAse L) that degrades viral RNA. Other genes activated by IFN-α/β binding to its receptor also contribute to the inhibition of viral replication. For example, IFN-α/β binding induces a specific protein kinase called dsRNA-dependent protein kinase (PKR), which inactivates protein synthesis, thus blocking viral replication in infected cells (Figure 17-2).

The binding of IFN-α and IFN-β to NK cells induces lytic activity, making them very effective in killing virally infected cells. The activity of NK cells is also greatly enhanced by IL-12, a cytokine that is produced very early in a response to viral infection.

Many Viruses are Neutralized by Antibodies

Antibodies specific for viral surface antigens are often crucial in containing the spread of a virus during acute infection and in protecting against reinfection. Antibodies are particularly effective in protecting against infection if they are localized at the site of viral entry into the body. Most viruses express surface receptor molecules that enable them to initiate infection by binding to specific host-cell membrane molecules. For example, influenza virus binds to sialic acid residues in cell-membrane glycoproteins and glycolipids; rhinovirus binds to intercellular adhesion molecules (ICAMs); and Epstein-Barr virus binds to type 2 complement receptors on B cells. If antibody to the viral receptor is produced, it can block infection altogether by preventing the binding of viral particles to host cells. Secretory IgA in mucous secretions plays an important role in host defense against viruses by blocking viral attachment to mucosal epithelial cells. The advantage of the attenuated oral polio vaccine, considered in Chapter 18, is that it induces production of secretory IgA, which effectively blocks attachment of poliovirus along the gastrointestinal tract.

Viral neutralization by antibody sometimes involves mechanisms that operate after viral attachment to host cells. In some cases, antibodies may block viral penetration by binding to epitopes that are necessary to mediate fusion of the viral envelope with the plasma membrane. If the induced antibody is of a complement-activating isotype, lysis of enveloped virions can ensue. Antibody or complement can also agglutinate viral particles and function as an opsonizing agent to facilitate Fc- or C3b-receptor–mediated phagocytosis of the viral particles.

Cell-Mediated Immunity is Important for Viral Control and Clearance

Although antibodies have an important role in containing the spread of a virus in the acute phases of infection, they are not usually able to eliminate the virus once infection has occurred—particularly if the virus is capable of entering a latent state in which its DNA is integrated into host chromosomal DNA. Once an infection is established, cell-mediated immune mechanisms are most important in host defense. In general, $CD8^+$ T_C cells and $CD4^+$ T_H1 cells are the main components of cell-mediated antiviral defense, although in some cases $CD4^+$ T_C cells have also been implicated. Activated T_H1 cells produce a number of cytokines, including IL-2, IFN-γ, and TNF, that defend against viruses either directly or indirectly. IFN-γ acts directly by inducing an antiviral state in cells. IL-2 acts indirectly by assisting in the recruitment of CTL precursors into an effector population. Both IL-2 and IFN-γ activate NK cells, which play an important role in host defense during the first days of many viral infections until a specific CTL response develops.

In most viral infections, specific CTL activity arises within 3–4 days after infection, peaks by 7–10 days, and then declines. Within 7–10 days of primary infection, most virions have been eliminated, paralleling the development of CTLs. CTLs specific for the virus eliminate virus-infected self-cells and thus eliminate potential sources of new virus. The role of CTLs in defense against viruses is demonstrated by the ability of virus-specific CTLs to confer protection for the specific virus on nonimmune recipients by adoptive transfer. The viral specificity of the CTL as well can be demonstrated with

adoptive transfer: adoptive transfer of a CTL clone specific for influenza virus strain X protects mice against influenza virus X but not against influenza virus strain Y.

Viruses Can Evade Host Defense Mechanisms

Despite their restricted genome size, a number of viruses have been found to encode proteins that interfere at various levels with specific or nonspecific host defenses. Presumably, the advantage of such proteins is that they enable viruses to replicate more effectively amidst host antiviral defenses. As described above, the induction of IFN-α and IFN-β is a major innate defense against viral infection, but some viruses have developed strategies to evade the action of IFN-α/β. These include hepatitis C virus, which has been shown to overcome the antiviral effect of the interferons by blocking or inhibiting the action of PKR (see Figure 17-2).

Another mechanism for evading host responses, utilized in particular by herpes simplex viruses (HSV) is inhibition of antigen presentation by infected host cells. HSV-1 and HSV-2 both express an immediate-early protein (a protein synthesized shortly after viral replication) called ICP47, which very effectively inhibits the human transporter molecule needed for antigen processing (TAP; see Figure 8-8). Inhibition of TAP blocks antigen delivery to class I MHC receptors on HSV-infected cells, thus preventing presentation of viral antigen to $CD8^+$ T cells. This results in the trapping of empty class I MHC molecules in the endoplasmic reticulum and effectively shuts down a $CD8^+$ T-cell response to HSV-infected cells.

The targeting of MHC molecules is not unique to HSV. Other viruses have been shown to down-regulate class I MHC expression shortly after infection. Two of the best-characterized examples, the adenoviruses and cytomegalovirus (CMV), use distinct molecular mechanisms to reduce the surface expression of class I MHC molecules, again inhibiting antigen presentation to $CD8^+$ T cells. Some viruses—CMV, measles virus, and HIV—have been shown to reduce levels of class II MHC molecules on the cell surface, thus blocking the function of antigen-specific antiviral helper T cells.

Antibody-mediated destruction of viruses requires complement activation, resulting either in direct lysis of the viral particle or opsonization and elimination of the virus by phagocytic cells. A number of viruses have strategies for evading complement-mediated destruction. Vaccinia virus, for example, secretes a protein that binds to the C4b complement component, inhibiting the classical complement pathway; and herpes simplex viruses have a glycoprotein component that binds to the C3b complement component, inhibiting both the classical and alternative pathways.

A number of viruses escape immune attack by constantly changing their antigens. In the influenza virus, continual antigenic variation results in the frequent emergence of new infectious strains. The absence of protective immunity to these newly emerging strains leads to repeated epidemics of influenza. Antigenic variation among rhinoviruses, the causative agent of the common cold, is responsible for our inability to produce an effective vaccine for colds. Nowhere is antigenic variation greater than in the human immunodeficiency virus (HIV), the causative agent of AIDS. Estimates suggest that HIV accumulates mutations at a rate 65 times faster than does influenza virus. Because of the importance of AIDS, a section of Chapter 19 addresses this disease.

A large number of viruses evade the immune response by causing generalized immunosuppression. Among these are the paramyxoviruses that cause mumps, the measles virus, Epstein-Barr virus (EBV), cytomegalovirus, and HIV. In some cases, immunosuppression is caused by direct viral infection of lymphocytes or macrophages. The virus can then either directly destroy the immune cells by cytolytic mechanisms or alter their function. In other cases, immunosuppression is the result of a cytokine imbalance. For example, EBV produces a protein, called BCRF1, that is homologous to IL-10; like IL-10, BCRF1 suppresses cytokine production by the T_H1 subset, resulting in decreased levels of IL-2, TNF, and IFN-γ.

Influenza Has Been Responsible for Some of the Worst Pandemics in History

The influenza virus infects the upper respiratory tract and major central airways in humans, horses, birds, pigs, and even seals. In 1918–19, an influenza pandemic (worldwide epidemic) killed more than 20 million people, a toll surpassing the number of casualties in World War I. Some areas, such as Alaska and the Pacific Islands, lost more than half of their population during that pandemic.

PROPERTIES OF THE INFLUENZA VIRUS

Influenza viral particles, or virions, are roughly spherical or ovoid in shape, with an average diameter of 90–100 nm. The virions are surrounded by an outer envelope—a lipid bilayer acquired from the plasma membrane of the infected host cell during the process of budding. Inserted into the envelope are two glycoproteins, **hemagglutinin (HA)** and **neuraminidase (NA),** which form radiating projections that are visible in electron micrographs (Figure 17-3). The hemagglutinin projections, in the form of trimers, are responsible for the attachment of the virus to host cells. There are approximately 1000 hemagglutinin projections per influenza virion. The hemagglutinin trimer binds to sialic acid groups on host-cell glycoproteins and glycolipids by way of a conserved amino acid sequence that forms a small groove in the hemagglutinin molecule. Neuraminidase, as its name indicates, cleaves *N*-acetylneuraminic (sialic) acid from nascent viral glycoproteins and host-cell membrane glycoproteins, an activity that presumably facilitates viral budding from the infected host cell. Within the envelope, an inner layer of matrix protein surrounds the nucleocapsid, which consists of eight dif-

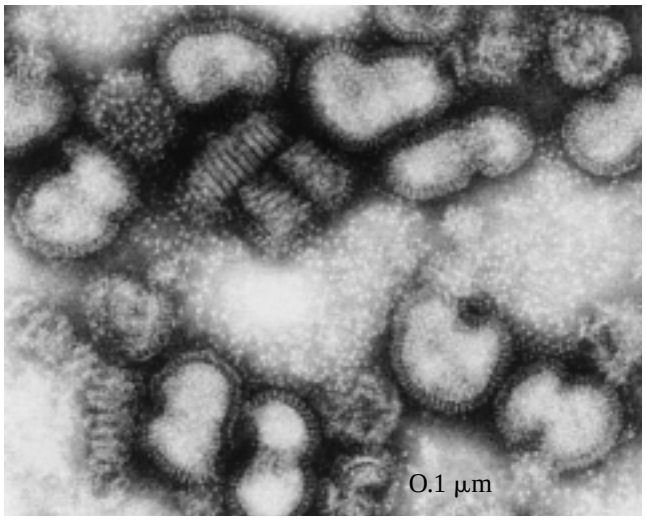


FIGURE 17-3 Electron micrograph of influenza virus reveals roughly spherical viral particles enclosed in a lipid bilayer with protruding hemagglutinin and neuraminidase glycoprotein spikes. *[Courtesy of G. Murti, Department of Virology, St. Jude Children's Research Hospital, Memphis, Tenn.]*

ferent strands of single-stranded RNA (ssRNA) associated with protein and RNA polymerase (Figure 17-4). Each RNA strand encodes one or more different influenza proteins.

Three basic types of influenza (A, B, and C), can be distinguished by differences in their nucleoprotein and matrix proteins. Type A, which is the most common, is responsible for the major human pandemics. Antigenic variation in hemagglutinin and neuraminidase distinguishes subtypes of type A influenza virus. According to the nomenclature of the World Health Organization, each virus strain is defined by its animal host of origin (specified, if other than human), geographical origin, strain number, year of isolation, and antigenic description of HA and NA (Table 17-2). For example, A/Sw/Iowa/15/30 (H1N1) designates strain-A isolate 15 that arose in swine in Iowa in 1930 and has antigenic subtypes 1 of HA and NA. Notice that the H and N spikes are antigenically distinct in these two strains. There are 13 different hemagglutinins and 9 neuraminidases among the type A influenza viruses.

The distinguishing feature of influenza virus is its variability. The virus can change its surface antigens so completely that the immune response to infection with the virus that caused a previous epidemic gives little or no protection against the virus causing a subsequent epidemic. The antigenic variation results primarily from changes in the hemagglutinin and neuraminidase spikes protruding from the viral envelope (Figure 17-5). Two different mechanisms generate antigenic variation in HA and NA: antigenic drift and antigenic shift. **Antigenic drift** involves a series of spontaneous point mutations that occur gradually, resulting in minor changes in HA and NA. **Antigenic shift** results in the sudden emergence of a new subtype of influenza whose HA and possibly also NA are considerably different from that of the virus present in a preceding epidemic.

The first time a human influenza virus was isolated was in 1934; this virus was given the subtype designation H0N1 (where H is hemagglutinin and N is neuraminidase). The H0N1 subtype persisted until 1947, when a major antigenic shift generated a new subtype, H1N1, which supplanted the previous subtype and became prevalent worldwide until 1957, when H2N2 emerged. The H2N2 subtype prevailed for the next decade and was replaced in 1968 by H3N2. Antigenic shift in 1977 saw the re-emergence of H1N1. The most recent antigenic shift, in 1989, brought the re-emergence of H3N2, which remained dominant throughout the next several years. However, an H1N1 strain re-emerged in Texas in 1995, and current influenza vaccines contain both H3N2 and H1N1 strains. With each antigenic shift, hemagglutinin and neuraminidase undergo major sequence changes, resulting in major antigenic variations for which the immune system lacks memory. Thus, each antigenic shift finds the population immunologically unprepared, resulting in major outbreaks of influenza, which sometimes reach pandemic proportions.

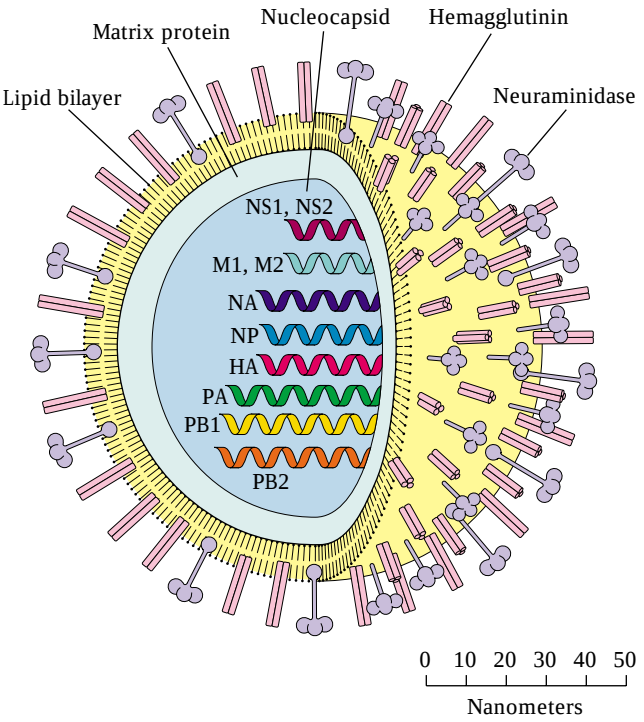


FIGURE 17-4 Schematic representation of influenza structure. The envelope is covered with neuraminidase and hemagglutinin spikes. Inside is an inner layer of matrix protein surrounding the nucleocapsid, which consists of eight ssRNA molecules associated with nucleoprotein. The eight RNA strands encode ten proteins: PB1, PB2, PA, HA (hemagglutinin), NP (nucleoprotein), NA (neuraminidase), M1, M2, NS1, and NS2.

TABLE 17-2 Some influenza A strains and their hemagglutinin (H) and neuraminidase (N) subtype

Species	Virus strain designation	Antigenic subtype
Human	A/Puerto Rico/8/34	H0N1
	A/Fort Monmouth/1/47	H1N1
	A/Singapore/1/57	H2N2
	A/Hong Kong/1/68	H3N2
	A/USSR/80/77	H1N1
	A/Brazil/11/78	H1N1
	A/Bangkok/1/79	H3N2
	A/Taiwan/1/86	H1N1
	A/Shanghai/16/89	H3N2
	A/Johannesburg/33/95	H3N2
	A/Wuhan/359/95	H3N2
	A/Texas/36/95	H1N1
	A/Hong Kong/156/97	H5N1
Swine	A/Sw/Iowa/15/30	H1N1
	A/Sw/Taiwan/70	H3N2
Horse (equine)	A/Eq/Prague/1/56	H7N7
	A/Eq/Miami/1/63	H3N8
Birds	A/Fowl/Dutch/27	H7N7
	A/Tern/South America/61	H5N3
	A/Turkey/Ontario/68	H8N4
	A/Chicken/Hong Kong/258/97	H5N1

Between pandemic-causing antigenic shifts, the influenza virus undergoes antigenic drift, generating minor antigenic variations, which account for strain differences within a subtype. The immune response contributes to the emergence of these different influenza strains. As individuals infected with a given influenza strain mount an effective immune response, the strain is eliminated. However, the accumulation of point mutations sufficiently alters the antigenicity of some variants so that they are able to escape immune elimination (Figure 17-6a). These variants become a new strain of influenza, causing another local epidemic cycle. The role of antibody in such immunologic selection can be demonstrated in the laboratory by mixing an influenza strain with a monoclonal antibody specific for that strain and then culturing the virus in cells. The antibody neutralizes all unaltered viral particles and only those viral particles with mutations resulting in altered antigenicity escape neutralization and are able to continue the infection. Within a short time in culture, a new influenza strain can be shown to emerge.

Antigenic shift is thought to occur through genetic reassortment between influenza virions from humans and from various animals, including horses, pigs, and ducks (Figure 17-6b). The fact that influenza contains eight separate strands of ssRNA makes possible the reassortment of the RNA strands of human and animal virions within a single cell infected with both viruses. Evidence for in vivo genetic reassortment between influenza A viruses from humans and domestic pigs was obtained in 1971. After infecting a pig simultaneously with human Hong Kong influenza (H3N2) and with swine influenza (H1N1), investigators were able to recover virions expressing H3N1. In some cases, an apparent antigenic shift may represent the re-emergence of a previous strain that has remained hidden for several decades. In May of 1977, a strain of influenza, A/USSR/77 (H1N1), appeared that proved to be identical to a strain that had caused an epidemic 27 years earlier. The virus could have been preserved over the years in a frozen state or in an animal reservoir. When such a re-emergence occurs, the HA and NA antigens expressed are not really new; however, they will be seen by the immune system of anyone not previously exposed to that strain (people under the age of twenty-seven in the 1977 epidemic, for example) as if they were new because no memory cells specific for these antigenic subtypes will exist in the susceptible population. Thus, from an immunologic point of view, the re-emergence of an old influenza A strain

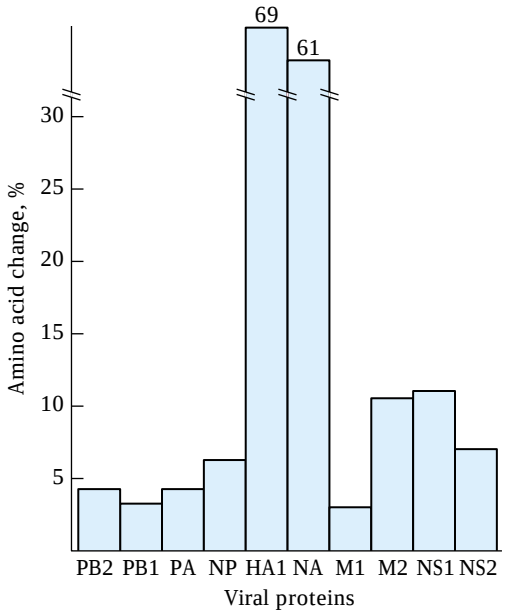


FIGURE 17-5 Amino acid sequence variation in 10 influenza viral proteins from two H3N2 strains and one H1N1 strain. The surface glycoproteins hemagglutinin (HA1) and neuraminidase (NA) show significant sequence variation; in contrast, the sequences of internal viral proteins, such as matrix proteins (M1 and M2) and nucleoprotein (NP), are largely conserved. *[Adapted from G. G. Brownlee, 1986, in* Options for the Control of Influenza, *Alan R. Liss.]*

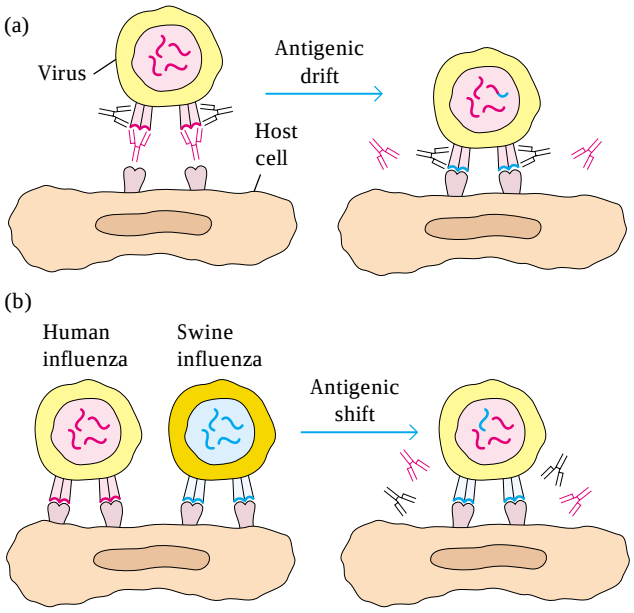


FIGURE 17-6 Two mechanisms generate variations in influenza surface antigens. (a) In antigenic drift, the accumulation of point mutations eventually yields a variant protein that is no longer recognized by antibody to the original antigen. (b) Antigenic shift may occur by reassortment of an entire ssRNA between human and animal virions infecting the same cell. Only four of the eight RNA strands are depicted.

can have the same effect as an antigenic shift that generates a new subtype.

HOST RESPONSE TO INFLUENZA INFECTION

Humoral antibody specific for the HA molecule is produced during an influenza infection. This antibody confers protection against influenza, but its specificity is strain-specific and is readily bypassed by antigenic drift. Antigenic drift in the HA molecule results in amino acid substitutions in several antigenic domains at the molecule's distal end (Figure 17-7). Two of these domains are on either side of the conserved sialic-acid–binding cleft, which is necessary for binding of virions to target cells. Serum antibodies specific for these two regions are important in blocking initial viral infectivity. These antibody titers peak within a few days of infection and then decrease over the next 6 months; the titers then plateau and remain fairly stable for the next several years. This antibody does not appear to be required for recovery from influenza, as patients with agammaglobulinemia recover from the disease. Instead, the serum antibody appears to play a significant role in resistance to reinfection by the same strain. When serum-antibody levels are high for a particular HA molecule, both mice and humans are resistant to infection by virions expressing that HA molecule. If mice are infected with influenza virus and antibody production is experimentally suppressed, the mice recover from the infection but can be reinfected with the same viral strain. In addition to humoral responses, CTLs can play a role in immune responses to influenza.

Bacterial Infections

Immunity to bacterial infections is achieved by means of antibody unless the bacterium is capable of intracellular growth, in which case delayed-type hypersensitivity has an important role. Bacteria enter the body either through a number of natural routes (e.g., the respiratory tract, the gastrointestinal tract, and the genitourinary tract) or through normally inaccessible routes opened up by breaks in mucous membranes or skin. Depending on the number of organisms

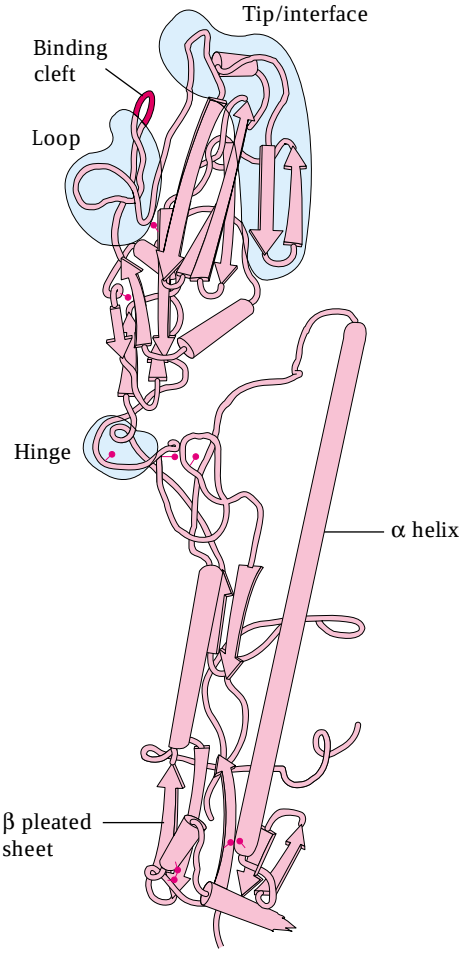


FIGURE 17-7 Structure of hemagglutinin molecule. Sialic acid on host cells interacts with the binding cleft, which is bounded by regions—designated the loop and tip/interface—where antigenic drift is prevalent (blue areas). Antibodies to these regions are important in blocking viral infections. Continual changes in amino acid residues in these regions allow the influenza virus to evade the antibody response. Small red dots represent residues that exhibit a high degree of variation among virus strains. *[Adapted from D. C. Wiley et al., 1981,* Nature ***289:****373.]*

Go to www.whfreeman.com/immunology Molecular Visualization
Viral Antigens *See Introduction and Flu Virus Hemagglutinin.*

entering and their virulence, different levels of host defense are enlisted. If the inoculum size and the virulence are both low, then localized tissue phagocytes may be able to eliminate the bacteria with an innate, nonspecific defense. Larger inoculums or organisms with greater virulence tend to induce an adaptive, specific immune response.

Immune Responses to Extracellular and Intracellular Bacteria Can Differ

Infection by extracellular bacteria induces production of humoral antibodies, which are ordinarily secreted by plasma cells in regional lymph nodes and the submucosa of the respiratory and gastrointestinal tracts. The humoral immune response is the main protective response against extracellular bacteria. The antibodies act in several ways to protect the host from the invading organisms, including removal of the bacteria and inactivation of bacterial toxins (Figure 17-8). Extracellular bacteria can be pathogenic because they induce a localized inflammatory response or because they produce toxins. The toxins, endotoxin or exotoxin, can be cytotoxic but also may cause pathogenesis in other ways. An excellent example of this is the toxin produced by diphtheria, which exerts a toxic effect on the cell by blocking protein synthesis. Endotoxins, such as lipopolysaccharides (LPS), are generally components of bacterial cell walls, while exotoxins, such as diphtheria toxin, are secreted by the bacteria.

Antibody that binds to accessible antigens on the surface of a bacterium can, together with the C3b component of complement, act as an opsonin that increases phagocytosis and thus clearance of the bacterium (see Figure 17-8). In the case of some bacteria—notably, the gram-negative organisms—complement activation can lead directly to lysis of the organism. Antibody-mediated activation of the complement system can also induce localized production of immune effector molecules that help to develop an amplified and more effective inflammatory response. For example, the complement split products C3a, C4a, and C5a act as anaphylatoxins, inducing local mast-cell degranulation and thus vasodilation and the extravasation of lymphocytes and neutrophils from the blood into tissue space (see Figure 17-8). Other complement split products serve as chemotactic factors for neutrophils and macrophages, thereby contributing to the buildup of phagocytic cells at the site of infection. Antibody to a bacteria toxin may bind to the toxin and neutralize it; the antibody-toxin complexes are then cleared by phagocytic cells in the same manner as any other antigen-antibody complex.

While innate immunity is not very effective against intracellular bacterial pathogens, intracellular bacteria can activate NK cells, which, in turn, provide an early defense against these bacteria. Intracellular bacterial infections tend to induce a cell-mediated immune response, specifically, delayed-type hypersensitivity. In this response, cytokines secreted by $CD4^+$ T cells are important—notably IFN-γ, which activates macrophages to kill ingested pathogens more effectively (see Figure 14-15).

Bacteria Can Effectively Evade Host Defense Mechanisms

There are four primary steps in bacterial infection:

- Attachment to host cells
- Proliferation
- Invasion of host tissue
- Toxin-induced damage to host cells

Host-defense mechanisms act at each of these steps, and many bacteria have evolved ways to circumvent some of these host defenses (Table 17-3).

Some bacteria have surface structures or molecules that enhance their ability to attach to host cells. A number of gram-negative bacteria, for instance, have pili (long hairlike projections), which enable them to attach to the membrane of the intestinal or genitourinary tract (Figure 17-9). Other bacteria, such as *Bordetella pertussis*, secrete adhesion molecules that attach to both the bacterium and the ciliated epithelial cells of the upper respiratory tract.

Secretory IgA antibodies specific for such bacterial structures can block bacterial attachment to mucosal epithelial cells and are the main host defense against bacterial attachment. However, some bacteria (e.g., *Neisseria gonorrhoeae*, *Haemophilus influenzae*, and *Neisseria meningitidis*) evade the IgA response by secreting proteases that cleave secretory IgA at the hinge region; the resulting Fab and Fc fragments have a shortened half-life in mucous secretions and are not able to agglutinate microorganisms.

Some bacteria evade the IgA response of the host by changing these surface antigens. In *N. gonorrhoeae*, for example, pilin, the protein component of the pili, has a highly variable structure. Variation in the pilin amino acid sequence is generated by gene rearrangements of its coding sequence. The pilin locus consists of one or two expressed genes and 10–20 silent genes. Each gene is arranged into six regions called *minicassettes*. Pilin variation is generated by a process of gene conversion, in which one or more minicassettes from the silent genes replace a minicassette of the expression gene. This process generates enormous antigenic variation, which may contribute to the pathogenicity of *N. gonorrhoeae* by increasing the likelihood that expressed pili will bind firmly to epithelial cells. In addition, the continual changes in the pilin sequence allow the organism to evade neutralization by IgA.

Some bacteria possess surface structures that serve to inhibit phagocytosis. A classic example is *Streptococcus pneumoniae*, whose polysaccharide capsule prevents phagocytosis very effectively. There are 84 serotypes of *S. pneumoniae* that differ from one another by distinct capsular polysaccharides.

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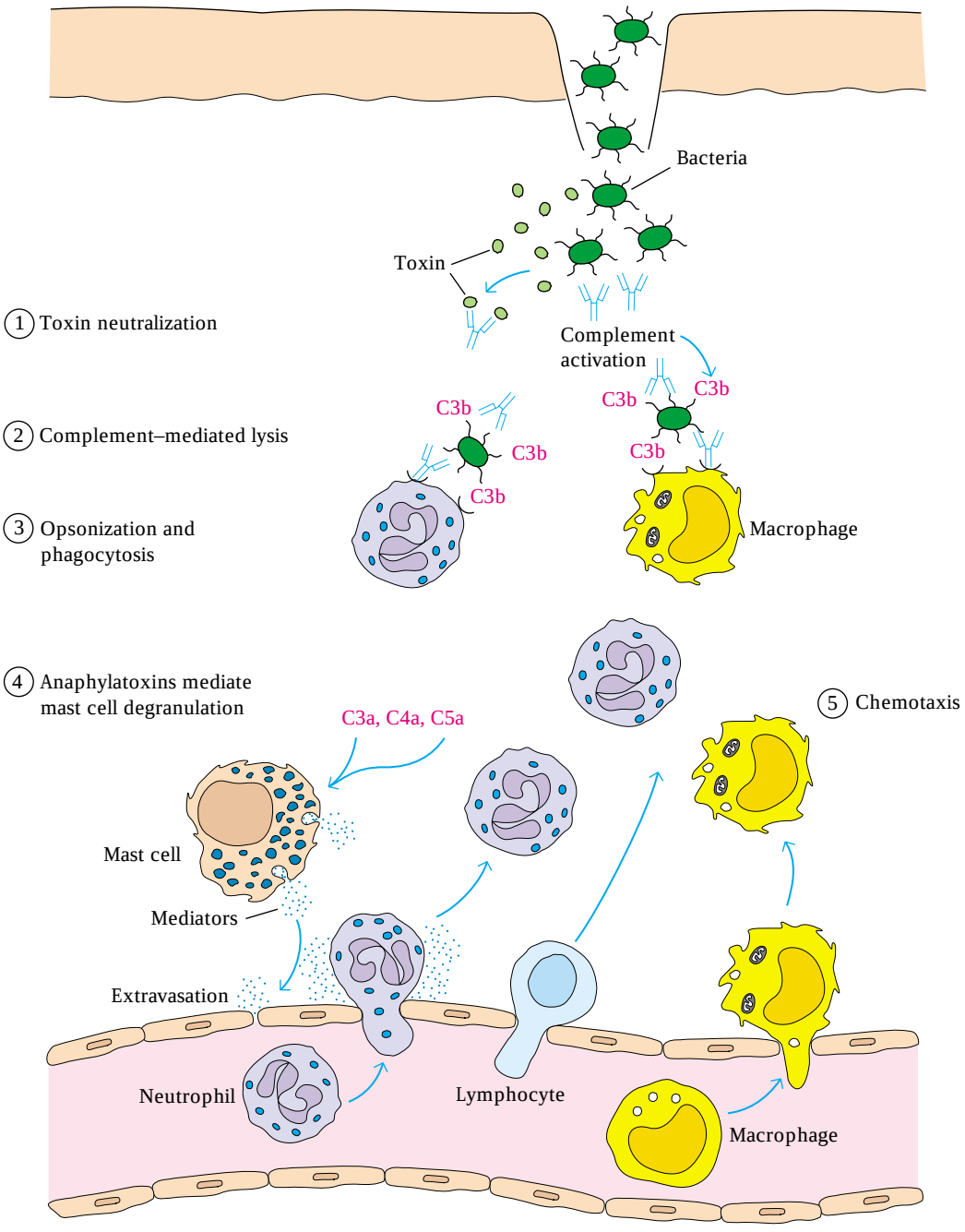

FIGURE 17-8 Antibody-mediated mechanisms for combating infection by extracellular bacteria. (1) Antibody neutralizes bacterial toxins. (2) Complement activation on bacterial surfaces leads to complement-mediated lysis of bacteria. (3) Antibody and the complement split product C_3b bind to bacteria, serving as opsonins to increase phagocytosis. (4) C3a, C4a, and C5a, generated by antibody-initiated complement activation, induce local mast cell degranulation, releasing substances that mediate vasodilation and extravasation of lymphocytes and neutrophils. (5) Other complement split products are chemotactic for neutrophils and macrophages.

TABLE 17-3 Host immune responses to bacterial infection and bacterial evasion mechanisms

Infection process	Host defense	Bacterial evasion mechanisms
Attachment to host cells	Blockage of attachment by secretory IgA antibodies	Secretion of proteases that cleave secretory IgA dimers (*Neisseria meningitidis, N. gonorrhoeae, Haemophilus influenzae*)
		Antigenic variation in attachment structures (pili of *N. gonorrhoeae*)
Proliferation	Phagocytosis (Ab- and C3b-mediated opsonization)	Production of surface structures (polysaccharide capsule, M protein, fibrin coat) that inhibit phagocytic cells
		Mechanisms for surviving within phagocytic cells
		Induction of apoptosis in macrophages (*Shigella flexneri*)
	Complement-mediated lysis and localized inflammatory response	Generalized resistance of gram-positive bacteria to complement-mediated lysis
		Insertion of membrane-attack complex prevented by long side chain in cell-wall LPS (some gram-negative bacteria)
Invasion of host tissues	Ab-mediated agglutination	Secretion of elastase that inactivates C3a and C5a (*Pseudomonas*)
Toxin-induced damage to host cells	Neturalization of toxin by antibody	Secretion of hyaluronidase, which enhances bacterial invasiveness

During infection, the host produces antibody against the infecting serotype. This antibody protects against reinfection with the same serotype but will not protect against infection by a different serotype. In this way, *S. pneumoniae* can cause disease many times in the same individual. On other bacteria, such as *Streptococcus pyogenes*, a surface protein projection called the M protein inhibits phagocytosis. Some pathogenic staphylococci are able to assemble a protective coat from host proteins. These bacteria secrete a coagulase enzyme that precipitates a fibrin coat around them, shielding them from phagocytic cells.

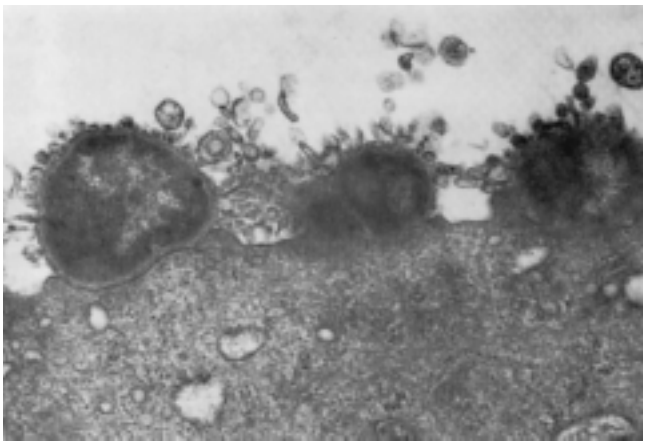

FIGURE 17-9 Electron micrograph of *Neisseria gonorrhoeae* attaching to urethral epithelial cells. Pili (P) extend from the gonococcal surface and mediate the attachment. *[From M. E. Ward and P. J. Watt, 1972,* J. Inf. Dis. ***126****:601.]*

Mechanisms for interfering with the complement system help other bacteria survive. In some gram-negative bacteria, for example, long side chains on the lipid A moiety of the cell-wall core polysaccharide help to resist complement-mediated lysis. *Pseudomonas* secretes an enzyme, elastase, that inactivates both the C3a and C5a anaphylatoxins, thereby diminishing the localized inflammatory reaction.

A number of bacteria escape host defense mechanisms by their ability to survive within phagocytic cells. Some, such as *Listeria monocytogenes*, do this by escaping from the phagolysosome to the cytoplasm, which is a more favorable environment for their growth. Other bacteria, such as *Mycobacterium avium*, block lysosomal fusion with the phagolysosome; and some mycobacteria are resistant to the oxidative attack that takes place within the phagolysosome.

Immune Responses Can Contribute to Bacterial Pathogenesis

In some cases, disease is caused not by the bacterial pathogen itself but by the immune response to the pathogen. As described in Chapter 12, pathogen-stimulated overproduction of cytokines leads to the symptoms of bacterial septic shock, food poisoning, and toxic-shock syndrome. For instance, cell-wall endotoxins of some gram-negative bacteria activate macrophages, resulting in release of high levels of IL-1 and TNF-α, which can cause septic shock. In staphylococcal food poisoning and toxic-shock syndrome, exotoxins produced by the pathogens function as superantigens, which can activate all T cells that express T-cell receptors with a particular V_β domain (see Table 10-4). The resulting overproduction of cytokines by activated T_H cells causes many of the symptoms of these diseases.

The ability of some bacteria to survive intracellularly within infected cells can result in chronic antigenic activation of $CD4^+$ T cells, leading to tissue destruction by a cell-mediated response with the characteristics of a delayed-type hypersensitivity reaction (see Chapter 14). Cytokines secreted by these activated $CD4^+$ T cells can lead to extensive accumulation and activation of macrophages, resulting in formation of a **granuloma.** The localized concentrations of lysosomal enzymes in these granulomas can cause extensive tissue necrosis. Much of the tissue damage seen with *M. tuberculosis* is due to a cell-mediated immune response.

Diphtheria (*Corynebacterium diphtheriae*) May Be Controlled by Immunization with Inactivated Toxoid

Diphtheria is the classic example of a bacterial disease caused by a secreted exotoxin to which immunity can be induced by immunization with an inactivated **toxoid.** The causative agent, a gram-positive, rodlike organism called *Corynebacterium diphtheriae*, was first described by Klebs in 1883 and was shown a year later by Loeffler to cause diphtheria in guinea pigs and rabbits. Autopsies on the infected animals revealed that, while bacterial growth was limited to the site of inoculation, there was widespread damage to a variety of organs, including the heart, liver, and kidneys. This finding led Loeffler to speculate that the neurologic and cardiologic manifestations of the disease were caused by a toxic substance elaborated by the organism.

Loeffler's hypothesis was validated in 1888, when Roux and Yersin produced the disease in animals by injecting them with a sterile filtrate from a culture of *C. diphtheriae.* Two years later, von Behring showed that an antiserum to the toxin was able to prevent death in infected animals. He prepared a toxoid by treating the toxin with iodine trichloride and demonstrated that it could induce protective antibodies in animals. However, the toxoid was still quite toxic and therefore unsuitable for use in humans. In 1923, Ramon found that exposing the toxin to heat and formalin rendered it nontoxic but did not destroy its antigenicity. Clinical trials showed that formaldehyde-treated toxoid conferred a high level of protection against diphtheria.

As immunizations with the toxoid increased, the number of cases of diphtheria decreased rapidly. In the 1920s, there were approximately 200 cases of diphtheria per 100,000 population in the United States. In 1989, the Centers for Disease Control reported only three cases of diphtheria in the entire United States. Recently in the former Soviet Union, there has been an alarming epidemic of diphtheria due to a reduction in vaccinations.

Natural infection with *C. diphtheriae* occurs only in humans. The disease is spread from one individual to another by airborne respiratory droplets. The organism colonizes the nasopharyngeal tract, remaining in the superficial layers of the respiratory mucosa. Growth of the organism itself causes little tissue damage, and only a mild inflammatory reaction develops. The virulence of the organism is due completely to its potent exotoxin. The toxin causes destruction of the underlying tissue, resulting in the formation of a tough fibrinous membrane ("pseudomembrane") composed of fibrin, white blood cells, and dead respiratory epithelial cells. The membrane itself can cause suffocation. The exotoxin also is responsible for widespread systemic manifestations. Pronounced myocardial damage (often leading to congestive heart failure) and neurologic damage (ranging from mild weakness to complete paralysis) are common.

The exotoxin that causes diphtheria symptoms is encoded by the *tox* gene carried by phage β. Within some strains of *C. diphtheriae*, phage β can exist in a state of **lysogeny,** in which the β-prophage DNA persists within the bacterial cell. Only strains that carry lysogenic phage β are able to produce the exotoxin. The diphtheria exotoxin contains two disulfide-linked chains, a binding chain and toxin chain. The binding chain interacts with ganglioside receptors on susceptible cells, facilitating internalization of the exotoxin. Toxicity results from the inhibitory effect of the toxin chain on protein synthesis. The diphtheria exotoxin is extremely potent; a single molecule has been shown to kill a cell. Removal of the binding chain prevents the exotoxin from entering the cell, thus rendering the exotoxin nontoxic. As described in Chapter 4, an immunotoxin can be prepared by replacing the binding chain with a monoclonal antibody specific for a tumor-cell surface antigen; in this way the toxin chain can be targeted to tumor cells (see Figure 4-23).

Today, diphtheria toxoid is prepared by treating diphtheria toxin with formaldehyde. The reaction with formaldehyde cross-links the toxin, resulting in an irreversible loss in its toxicity while enhancing its antigenicity. The toxoid is administered together with tetanus toxoid and inactivated *Bordetella pertussis* in a combined vaccine that is given to children beginning at 6–8 weeks of age. Immunization with the toxoid induces the production of antibodies (antitoxin), which can bind to the toxin and neutralize its activity. Because antitoxin levels decline slowly over time, booster doses are recommended at 10-year intervals to maintain antitoxin levels within the protective range. Interestingly, antibodies specific for epitopes on the binding chain of the diphtheria exotoxin are critical for toxin neutralization because these antibodies block internalization of the active toxin chain.

Tuberculosis (*Mycobacterium tuberculosis*) Is Primarily Controlled by $CD4^+$ T Cells

Tuberculosis is the leading cause of death in the world from a single infectious agent, killing about 3 million individuals every year and accounting for 18.5% of all deaths in adults between the ages of 15 and 59. About 1.79 billion people, roughly one-third of the world's population, are infected with the causative agent *M. tuberculosis* and are at risk of developing the disease. Long thought to have been eliminated as a

public health problem in the United States, tuberculosis re-emerged in the early 1990s, particularly in the inner cities and in areas where HIV-infection levels are high (see the last section of this chapter). In 2000, approximately 17,000 individuals were diagnosed with tuberculosis in the United States.

Although several *Mycobacterium* species can cause tuberculosis, *M. tuberculosis* is the principal causative agent. This organism is spread easily, and pulmonary infection usually results from inhalation of small droplets of respiratory secretions containing a few bacilli. The inhaled bacilli are ingested by alveolar macrophages and are able to survive and multiply intracellularly by inhibiting formation of phagolysosomes. When the infected macrophages lyse, as they eventually do, large numbers of bacilli are released. A cell-mediated response involving $CD4^+$ T cells, which is required for immunity to tuberculosis, may be responsible for much of the tissue damage in the disease. $CD4^+$ T-cell activity is the basis for the tuberculin skin test to the purified protein derivative (PPD) from *M. tuberculosis* (see Chapter 14).

Upon infection with *M. tuberculosis,* the most common clinical pattern, termed pulmonary tuberculosis, appears in about 90% of those infected. In this pattern, $CD4^+$ T cells are activated within 2–6 weeks after infection, inducing the infiltration of large numbers of activated macrophages. These cells wall off the organism inside a granulomatous lesion called a tubercle (Figure 17-10). A tubercle consists of a few small lymphocytes and a compact collection of activated macrophages, which sometimes differentiate into epithelioid cells or multinucleated giant cells. The massive activation of macrophages that occurs within tubercles often results in the concentrated release of lytic enzymes. These enzymes destroy nearby healthy cells, resulting in circular regions of necrotic tissue, which eventually form a lesion with a caseous (cheese-like) consistency (see Figure 17-10). As these caseous lesions heal, they become calcified and are readily visible on x-rays, where they are called Ghon complexes.

Because the activated macrophages suppress proliferation of the phagocytosed bacilli, infection is contained. Cytokines produced by $CD4^+$ T cells (T_H1 subset) play an important role in the response by activating macrophages, so that they are able to kill the bacilli or inhibit their growth. The role of IFN-γ in the immune response to mycobacteria has been demonstrated with knockout mice lacking IFN-γ. These mice died when they were infected with an attenuated strain of mycobacteria (BCG), whereas IFN-γ^+ normal mice survive.

Recent studies have revealed high levels of IL-12 in the pleural effusions of tuberculosis patients. The high levels of IL-12, produced by activated macrophages, are not surprising, given the decisive role of IL-12 in stimulating T_H1-mediated responses (see Figure 12-12). In mouse models of tuberculosis, IL-12 has been shown to increase resistance to the disease. Not only does IL-12 stimulate development of T_H1 cells, but it also may contribute to resistance by inducing the production of chemokines that attract macrophages to the site of infection. When IL-12 is neutralized by antibody to IL-12, granuloma formation in tuberculous mice is blocked.

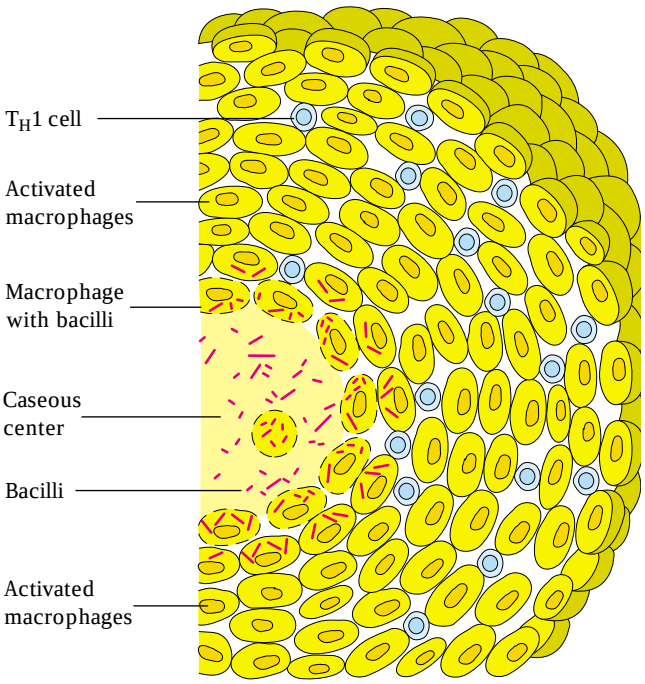

FIGURE 17-10 A tubercle formed in pulmonary tuberculosis. *[Modified from A. M. Dannenberg, 1993,* Hosp. Prac. *(Jan. 15):51.]*

The $CD4^+$ T-cell–mediated immune response mounted by the majority of people exposed to *M. tuberculosis* thus controls the infection and later protects against reinfection. However, about 10% of individuals infected with *M. tuberculosis* follow a different clinical pattern: the disease progresses to chronic pulmonary tuberculosis or extrapulmonary tuberculosis. This progression may occur years after the primary infection. In this clinical pattern, accumulation of large concentrations of mycobacterial antigens within tubercles leads to extensive and continual chronic $CD4^+$ T-cell activation and ensuing macrophage activation. The resulting high concentrations of lytic enzymes cause the necrotic caseous lesions to liquefy, creating a rich medium that allows the tubercle bacilli to proliferate extracellularly. Eventually the lesions rupture, and the bacilli disseminate in the lung and/or are spread through the blood and lymphatic vessels to the pleural cavity, bone, urogenital system, meninges, peritoneum, or skin.

Tuberculosis is treated with several drugs used in combination, including isoniazid, rifampin, streptomycin, pyrazinamide, and ethambutol. The combination therapy of isoniazid and rifampin has been particularly effective. The intracellular growth of *M. tuberculosis* makes it difficult for drugs to reach the bacilli. For this reason, drug therapy must be continued for at least 9 months to eradicate the bacteria. Some patients with tuberculosis do not exhibit any clinical symptoms, and some patients with symptoms begin to feel better within 2–4 weeks

after treatment begins. To avoid the side effects associated with the usual antibiotic therapy, many patients, once they feel better, stop taking the medications long before the recommended treatment period is completed. Because briefer treatment may not eradicate organisms that are somewhat resistant to the antibiotics, a multidrug-resistant strain can emerge. Noncompliance with required treatment regimes, one of the most troubling aspects of the large number of current tuberculosis cases, clearly compromises efforts to contain the spread of the disease.

Presently, the only vaccine for *M. tuberculosis* is an attenuated strain of *M. bovis* called BCG (Bacillus Calmette-Guerin). The vaccine appears to provide fairly effective protection against extrapulmonary tuberculosis but has been inconsistent against pulmonary tuberculosis. In different studies, BCG has provided protection in anywhere from 0% to 80% of vaccinated individuals; in some cases, BCG vaccination has even increased the risk of infection. Moreover, after BCG vaccination, the tuberculin skin test cannot be used as an effective monitor of exposure to *M. tuberculosis.* Because of the variable effectiveness of the BCG vaccine and the inability to monitor for exposure with the skin test after vaccination, this vaccine is not used in the United States. However, the alarming increase in multidrug-resistant strains has stimulated renewed efforts to develop a more effective tuberculosis vaccine.

Protozoan Diseases

Protozoans are unicellular eukaryotic organisms. They are responsible for several serious diseases in humans, including amoebiasis, Chagas' disease, African sleeping sickness, malaria, leishmaniasis, and toxoplasmosis. The type of immune response that develops to protozoan infection and the effectiveness of the response depend in part on the location of the parasite within the host. Many protozoans have life-cycle stages in which they are free within the bloodstream, and it is during these stages that humoral antibody is most effective. Many of these same pathogens are also capable of intracellular growth; during these stages, cell-mediated immune reactions are effective in host defense. In the development of vaccines for protozoan diseases, the branch of the immune system that is most likely to confer protection must be carefully considered.

Malaria (*Plasmodium* Species) Infects 600 Million People Worldwide

Malaria is one of the most devastating diseases in the world today, infecting nearly 10% of the world population and causing 1–2 million deaths every year. Malaria is caused by various species of the genus *Plasmodium*, of which *P. falciparum* is the most virulent and prevalent. The alarming development of multiple-drug resistance in *Plasmodium* and the increased resistance of its vector, the *Anopheles* mosquito, to DDT underscore the importance of developing new strategies to hinder the spread of malaria.

PLASMODIUM LIFE CYCLE AND PATHOGENESIS OF MALARIA

Plasmodium progresses through a remarkable series of developmental and maturational stages in its extremely complex life cycle. Female *Anopheles* mosquitoes, which feed on blood meals, serve as the vector for *Plasmodium*, and part of the parasite's life cycle takes place within the mosquito. (Because male *Anopheles* mosquitoes feed on plant juices, they do not transmit *Plasmodium*.)

Human infection begins when sporozoites, one of the *Plasmodium* stages, are introduced into an individual's bloodstream as an infected mosquito takes a blood meal (Figure 17-11). Within 30 min, the sporozoites disappear from the

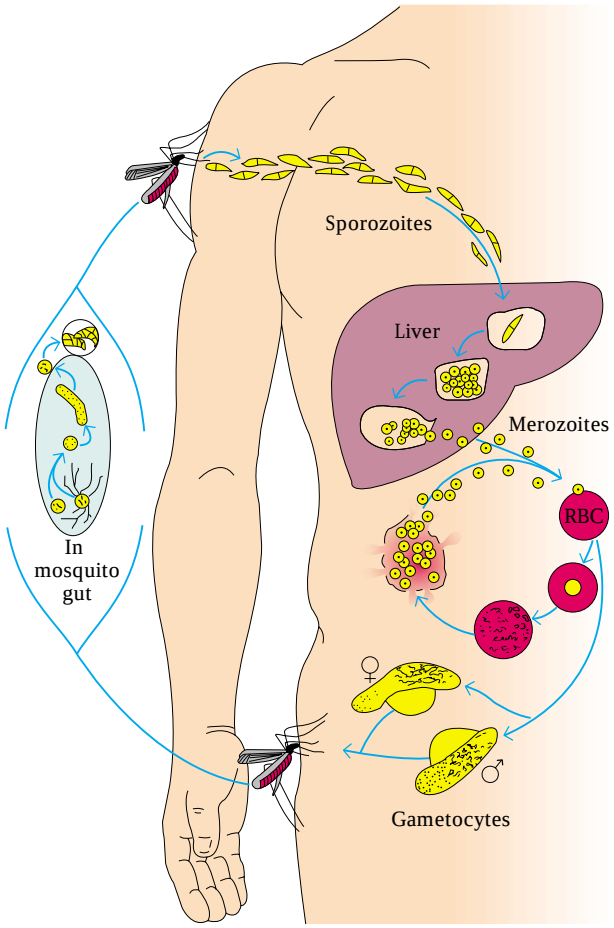

FIGURE 17-11 The life cycle of *Plasmodium.* Sporozoites enter the bloodstream when an infected mosquito takes a blood meal. The sporozoites migrate to the liver, where they multiply, transforming liver hepatocytes into giant multinucleate schizonts, which release thousands of merozoites into the bloodstream. The merozoites infect red blood cells, which eventually rupture, releasing more merozoites. Eventually some of the merozoites differentiate into male and female gametocytes, which are ingested by a mosquito and differentiate into gametes. The gametes fuse to form a zygote that differentiates to the sporozoite stage within the salivary gland of the mosquito.

blood as they migrate to the liver, where they infect hepatocytes. Sporozoites are long, slender cells that are covered by a 45-kDa protein called circumsporozoite (CS) antigen, which appears to mediate their adhesion to hepatocytes. The binding site on the CS antigen is a conserved region in the carboxyl-terminal end (called region II) that has a high degree of sequence homology with known cell-adhesion molecules.

Within the liver, the sporozoites multiply extensively and undergo a complex series of transformations that culminate in the formation and release of merozoites in about a week. It has been estimated that a liver hepatocyte infected with a single sporozoite can release 5,000–10,000 merozoites. The released merozoites infect red blood cells, initiating the symptoms and pathology of malaria. Within a red blood cell, merozoites replicate and undergo successive differentiations; eventually the cell ruptures and releases new merozoites, which go on to infect more red blood cells. Eventually some of the merozoites differentiate into male and female gametocytes, which may be ingested by a female *Anopheles* mosquito during a blood meal. Within the mosquito's gut, the male and female gametocytes differentiate into gametes that fuse to form a zygote, which multiplies and differentiates into sporozoites within the salivary gland. The infected mosquito is now set to initiate the cycle once again.

The symptoms of malaria are recurrent chills, fever, and sweating. The symptoms peak roughly every 48 h, when successive generations of merozoites are released from infected red blood cells. An infected individual eventually becomes weak and anemic and shows splenomegaly. The large numbers of merozoites formed can block capillaries, causing intense headaches, renal failure, heart failure, or cerebral damage—often with fatal consequences. There is speculation that some of the symptoms of malaria may be caused not by *Plasmodium* itself but instead by excessive production of cytokines. This hypothesis stemmed from the observation that cancer patients treated in clinical trials with recombinant tumor necrosis factor (TNF) developed symptoms that mimicked malaria. The relation between TNF and malaria symptoms was studied by infecting mice with a mouse-specific strain of *Plasmodium*, which causes rapid death by cerebral malaria. Injection of these mice with antibodies to TNF was shown to prevent the rapid death.

HOST RESPONSE TO PLASMODIUM INFECTION

In regions where malaria is endemic, the immune response to *Plasmodium* infection is poor. Children less than 14 years old mount the lowest immune response and consequently are most likely to develop malaria. In some regions, the childhood mortality rate for malaria reaches 50%, and worldwide the disease kills about a million children a year. The low immune response to *Plasmodium* among children can be demonstrated by measuring serum antibody levels to the sporozoite stage. Only 22% of the children living in endemic areas have detectable antibodies to the sporozoite stage, whereas 84% of the adults have such antibodies. Even in adults, the degree of immunity is far from complete, however, and most people living in endemic regions have lifelong low-level *Plasmodium* infections.

A number of factors may contribute to the low levels of immune responsiveness to *Plasmodium*. The maturational changes from sporozoite to merozoite to gametocyte allow the organism to keep changing its surface molecules, resulting in continual changes in the antigens seen by the immune system. The intracellular phases of the life cycle in liver cells and erythrocytes also reduce the degree of immune activation generated by the pathogen and allow the organism to multiply while it is shielded from attack. Furthermore, the most accessible stage, the sporozoite, circulates in the blood for only about 30 min before it infects liver hepatocytes; it is unlikely that much immune activation can occur in such a short period of time. And even when an antibody response does develop to sporozoites, *Plasmodium* has evolved a way of overcoming that response by sloughing off the surface CS-antigen coat, thus rendering the antibodies ineffective.

DESIGN OF MALARIA VACCINES

An effective vaccine for malaria should maximize the most effective immune defense mechanisms. Unfortunately, little is known of the roles that humoral and cell-mediated responses play in the development of protective immunity to this disease. Current approaches to design of malaria vaccines focus largely on the sporozoite stage. One experimental vaccine, for example, consists of *Plasmodium* sporozoites attenuated by x-irradiation. In one study, nine volunteers were repeatedly immunized by the bite of *P. falciparum*–infected, irradiated mosquitoes. Later challenge by the bites of mosquitoes infected with virulent *P. falciparum* revealed that six of the nine recipients were completely protected. These results are encouraging, but translating these findings into mass immunization remains problematic. Sporozoites do not grow well in cultured cells, so an enormous insectory would be required to breed mosquitoes in which to prepare enough irradiated sporozoites to vaccinate just one small village.

Current vaccine strategies are aimed at producing synthetic subunit vaccines consisting of epitopes that can be recognized by T cells and B cells. While no effective vaccine has been developed, this is an active area of investigation.

African Sleeping Sickness (*Trypanosoma* Species)

Two species of African trypanosomes, which are flagellated protozoans, can cause sleeping sickness, a chronic, debilitating disease transmitted to humans and cattle by the bite of the tsetse fly. In the bloodstream, a trypanosome differentiates into a long, slender form that continues to divide every 4–6 h. The disease progresses through several stages, beginning with an early (systemic) stage in which trypanosomes multiply in the blood and progressing to a neurologic stage in which the

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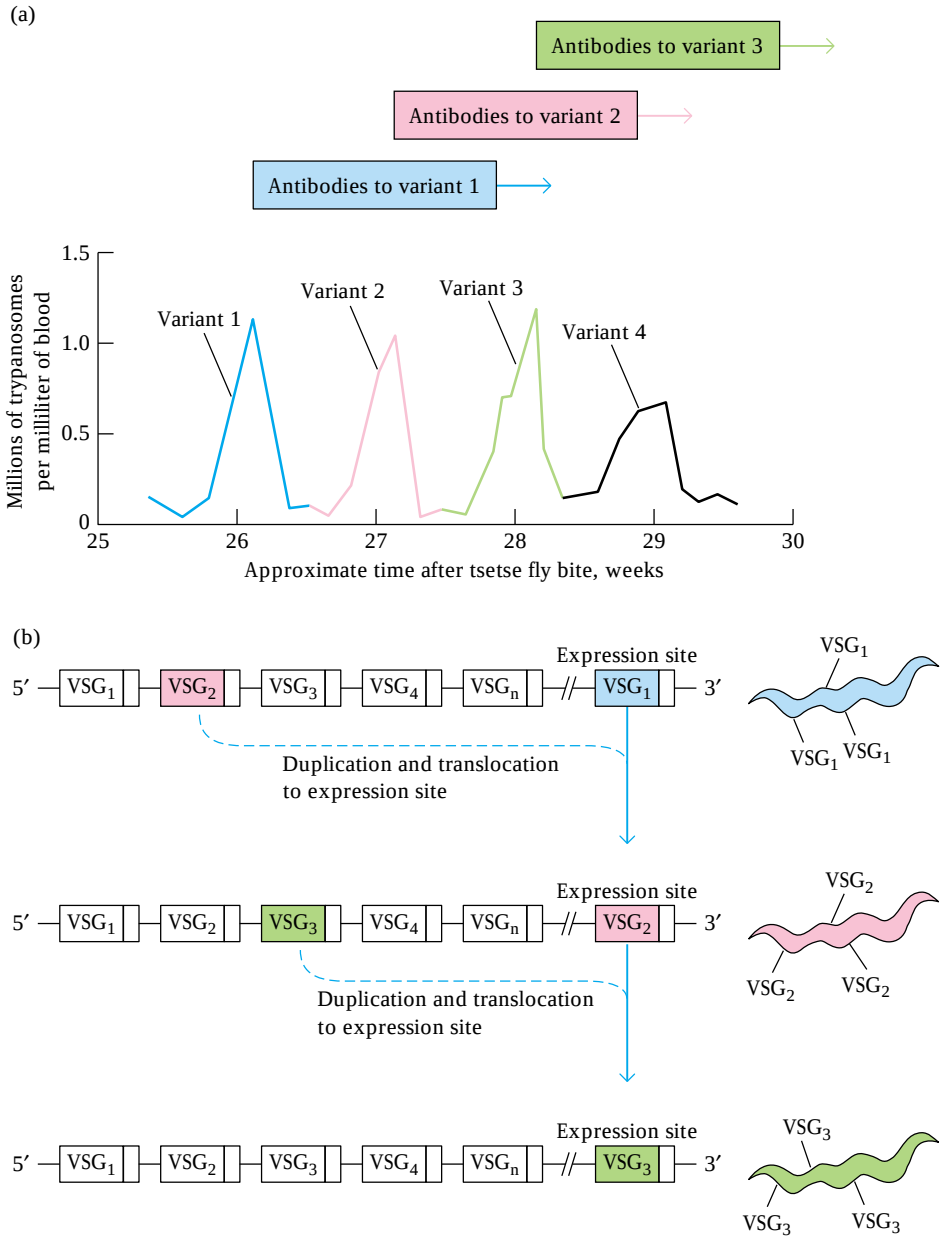

FIGURE 17-12 (a) Successive waves of parasitemia after infection with *Trypanosoma* result from antigenic shifts in the parasite's variant surface glycoprotein (VSG). Each variant that arises is unaffected by the humoral antibodies induced by the previous variant. (b) Antigenic shifts in trypanosomes occur by the duplication of gene segments encoding variant VSG molecules and their translocation to an expression site located close to the telomere. *[Part (a) adapted from J. Donelson, 1988,* The Biology of Parasitism, *Alan R. Liss.]*

parasite infects the central nervous system, causing meningoencephalitis and eventually the loss of consciousness.

As parasite numbers increase after infection, an effective humoral antibody response develops to the glycoprotein coat, called variant surface glycoprotein (VSG), that covers the trypanosomal surface (Figure 17-12). These antibodies eliminate most of the parasites from the bloodstream, both by complement-mediated lysis and by opsonization and

subsequent phagocytosis. However, about 1% of the organisms, which bear an antigenically different VSG, escape the initial antibody response. These surviving organisms now begin to proliferate in the bloodstream, and a new wave of parasitemia is observed. The successive waves of parasitemia reflect a unique mechanism of antigenic shift by which the trypanosomes can evade the immune response to their glycoprotein antigens. This process is so effective that each new variant that arises in the course of a single infection is able to escape the humoral antibodies generated in response to the preceding variant, so that waves of parasitemia recur (Figure 17-12a).

Several unusual genetic processes generate the extensive variation in trypanosomal VSG that enables the organism to escape immunologic clearance. An individual trypanosome carries a large repertoire of VSG genes, each encoding a different VSG primary sequence. *Trypanosoma brucei*, for example, contains more than 1000 VSG genes in its genome, clustered at multiple chromosomal sites. A trypanosome expresses only a single VSG gene at a time. Activation of a VSG gene results in duplication of the gene and its transposition to a transcriptionally active expression site (ES) at the telomeric end of specific chromosomes (Figure 17-12b). Activation of a new VSG gene displaces the previous gene from the telomeric expression site. A number of chromosomes in the trypanosome have transcriptionally active expression sites at the telomeric ends, so that a number of VSG genes can potentially be expressed, but unknown control mechanisms limit expression to a single VSG expression site at a time.

There appears to be some order in the VSG variation during infection. Each new variant arises not by clonal outgrowth from a single variant cell but instead from the growth of multiple cells that have activated the same VSG gene in the current wave of parasite growth. It is not known how this process is regulated among individual trypanosomes. The continual shifts in epitopes displayed by the VSG make the development of a vaccine for African sleeping sickness extremely difficult.

Leishmaniasis Is a Useful Model for Demonstrating Differences in Host Responses

The protozoan parasite *Leishmania major* provides a powerful and illustrative example of how host responses can differ between individuals. These differences can lead to either clearance of the parasite or fatality from the infection. *Leishmania* is a protozoan that lives in the phagosomes of macrophages. Resistance to the infection correlates well with the production of IFN-γ and the development of a T_H1 response. Elegant studies in mice have demonstrated that strains that are resistant to *Leishmania* develop a T_H1 response and produce IFN-γ upon infection. Such strains of mice become highly susceptible to *Leishmania*-induced fatality if they lose either IFN-γ or the IFN-γ receptor, further underscoring the importance of IFN-γ in containing the infection. A few strains of mice, such as BALB/c, are highly susceptible to *Leishmania*, and these animals frequently succumb to infection. These mice mount a T_H2-type response to *Leishmania* infection; they produce high levels of IL-4 and essentially no IFN-γ. Thus, one difference between an effective and an ineffective defense against the parasite is the development of a T_H1 response or a T_H2 response. Recent studies demonstrate that one difference between the resistant strains of mice and BALB/c is that a small restricted subset of BALB/c $CD4^+$ T cells are capable of recognizing a particular epitope on *L. major*, and this subset produces high levels of IL-4 early in the response to the parasite. This skews the response toward a predominantly T_H2 type. Understanding how these different T-helper responses affect the outcome of infection could provide a more rational approach to the design of effective treatments and successful vaccines for other pathogens.

Diseases Caused by Parasitic Worms (Helminths)

Unlike protozoans, which are unicellular and often grow within human cells, helminths are large, multicellular organisms that reside in humans but do not ordinarily multiply there and are not intracellular pathogens. Although helminths are more accessible to the immune system than protozoans, most infected individuals carry few of these parasites; for this reason, the immune system is not strongly engaged and the level of immunity generated to helminths is often very poor.

Parasitic worms are responsible for a wide variety of diseases in both humans and animals. More than a billion people are infected with *Ascaris*, a parasitic roundworm that infects the small intestine, and more than 300 million people are infected with *Schistosoma*, a trematode worm that causes a chronic debilitating infection. Several helminths are important pathogens of domestic animals and invade humans who ingest contaminated food. These helminths include *Taenia*, a tapeworm of cattle and pigs, and *Trichinella*, the roundworm of pigs that causes trichinosis.

Several *Schistosoma* species are responsible for the chronic, debilitating, and sometimes fatal disease **schistosomiasis** (formerly known as *bilharzia*). Three species, *S. mansoni*, *S. japonicum*, and *S. haematobium*, are the major pathogens in humans, infecting individuals in Africa, the Middle East, South America, the Caribbean, China, Southeast Asia, and the Philippines. A rise in the incidence of schistosomiasis in recent years has paralleled the increasing worldwide use of irrigation, which has expanded the habitat of the freshwater snail that serves as the intermediate host for schistosomes.

Infection occurs through contact with free-swimming infectious larvae, called cercariae, which are released from an infected snail at the rate of 300–3000 per day. When cercariae contact human skin, they secrete digestive enzymes that help them to bore into the skin, where they shed their tails and are transformed into schistosomules. The schistosomules enter the capillaries and migrate to the lungs, then to the liver, and finally to the primary site of infection, which varies with the species. *S. mansoni* and *S. japonicum* infect the intestinal mesenteric veins; *S. haematobium* infects the veins of the urinary bladder. Once established in their final tissue site, schistosomules mature into male and female adult worms. The worms mate and the females produce at least 300 spiny eggs a day. Unlike protozoan parasites, schistosomes and other helminths do not multiply within their hosts. The eggs produced by the female worm do not mature into adult worms in humans; instead, some of them pass into the feces or urine and are excreted to infect more snails. The number of worms in an infected individual increases only through repeated exposure to the free-swimming cercariae, and so most infected individuals carry rather low numbers of worms.

Most of the symptoms of schistosomiasis are initiated by the eggs. As many as half of the eggs produced remain in the host, where they invade the intestinal wall, liver, or bladder and cause hemorrhage. A chronic state can then develop in which the adult worms persist and the unexcreted eggs induce cell-mediated delayed-type hypersensitive reactions, resulting in large granulomas that are gradually walled off by fibrous tissue. Although the eggs are contained by the formation of the granuloma, often the granuloma itself obstructs the venous blood flow to the liver or bladder.

Although an immune response does develop to the schistosomes, in most individuals it is not sufficient to eliminate the adult worms, even though the intravascular sites of schistosome infestation should make the worm an easy target for immune attack. Instead, the worms survive for up to 20 years. The schistosomules would appear to be the forms most susceptible to attack, but because they are motile, they can evade the localized cellular buildup of immune and inflammatory cells. Adult schistosome worms also have several unique mechanisms that protect them from immune defenses. The adult worm has been shown to decrease the expression of antigens on its outer membrane and also to enclose itself in a glycolipid and glycoprotein coat derived from the host, masking the presence of its own antigens. Among the antigens observed on the adult worm are the host's own ABO blood-group antigens and histocompatibility antigens! The immune response is, of course, diminished by this covering made of the host's self-antigens, which must contribute to the lifelong persistence of these organisms.

The relative importance of the humoral and cell-mediated responses in protective immunity to schistosomiasis is controversial. The humoral response to infection with *S. mansoni* is characterized by high titers of antischistosome IgE antibodies, localized increases in mast cells and their subsequent degranulation, and increased numbers of eosinophils (Figure 17-13, *top*). These manifestations suggest that cytokines produced by a T_H2-like subset are important for the immune response: IL-4, which induces B cells to class-switch to IgE production; IL-5, which induces bone-marrow precursors to differentiate into eosinophils; and IL-3, which (along with IL-4) stimulates growth of mast cells. Degranulation of mast cells releases mediators that increase the infiltration of such inflammatory cells as macrophages and eosinophils. The eosinophils express Fc receptors for IgE and IgG and bind to the antibody-coated parasite. Once bound to the parasite, an eosinophil can participate in antibody-dependent cell-mediated cytotoxicity (ADCC), releasing mediators from its granules that damage the parasite (see Figure 14-12). One eosinophil mediator, called basic protein, is particularly toxic to helminths.

Immunization studies with mice, however, suggest that this humoral IgE response may not provide protective immunity. When mice are immunized with *S. mansoni* vaccine, the protective immune response that develops is not an IgE response, but rather a T_H1 response characterized by IFN-γ production and macrophage accumulation (Figure 17-13, *bottom*). Furthermore, inbred strains of mice with deficiencies in mast cells or IgE develop protective immunity from vaccination, whereas inbred strains with deficiencies in cell-mediated $CD4^+$ T-cell responses fail to develop protective immunity in response to the vaccine. These studies suggest that the $CD4^+$ T-cell response may be the most important in immunity to schistosomiasis. It has been suggested that the ability to induce an ineffective T_H2-like response may have evolved in schistosomes as a clever defense mechanism to ensure that T_H2 cells produced sufficient levels of IL-10 to inhibit protective immunity mediated by the T_H1-like subset in the $CD4^+$ T response.

Antigens present on the membrane of cercariae and young schistosomules are promising vaccine components because these stages appear to be most susceptible to immune attack. Injecting mice and rats with monoclonal antibodies to cercariae and young schistosomules passively transferred resistance to infection with live cercariae. When these protective antibodies were used in affinity columns to purify schistosome membrane antigens from crude membrane extracts, it was found that mice immunized and boosted with these purified antigens exhibited increased resistance to a later challenge with live cercariae. Schistosome cDNA libraries were then established and screened with the protective monoclonal antibodies to identify those cDNAs encoding surface antigens. Experiments using cloned cercariae or schistosomule antigens are presently under way to assess their ability to induce protective immunity in animal models. However, in developing an effective vaccine for schistosomiasis, a fine line separates a beneficial immune response, which at best limits the parasite load, from a detrimental response, which in itself may become pathologic.

VISUALIZING CONCEPTS

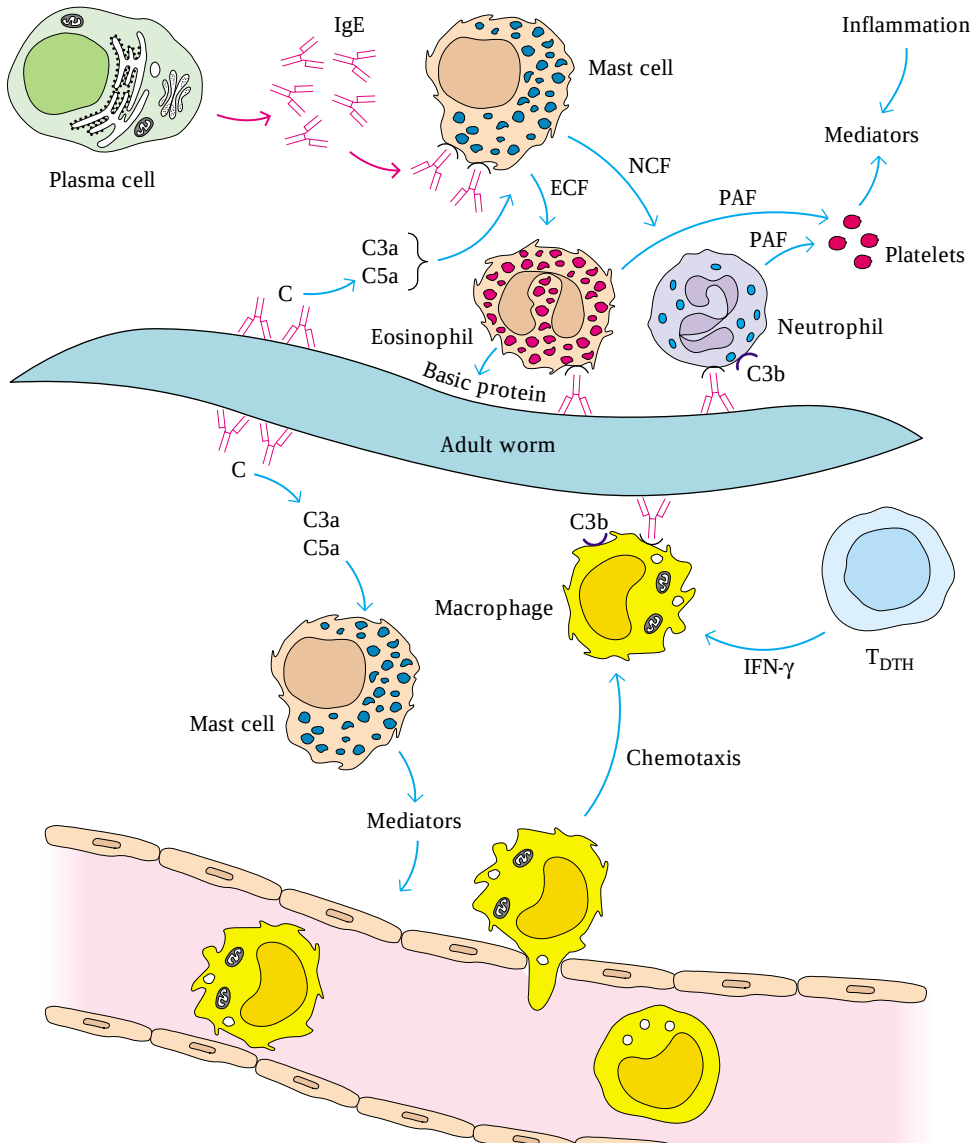

FIGURE 17-13 Overview of the immune response generated against *Schistosoma mansoni*. The response includes an IgE humoral component (*top*) and a cell-mediated component involving $CD4^+$ T cells (*bottom*). C = complement; ECF = eosinophil chemotactic factor; NCF = neutrophil chemotactic factor; PAF = platelet-activating factor.

Emerging Infectious Diseases

A cursory glance at the current offerings in your local bookstore or video rental store brings into focus the preoccupation of the public and the press with new infectious agents. Several times a year, it seems, we hear about a new virus or bacterium that arises in a particular location and causes severe illness or death in a population. Newly described pathogens are referred to as emerging pathogens. Some of the emerging pathogens that have been described since the early 1970s appear in Table 17-4. These new pathogens are thought to have emerged within the recent past. HIV is an example of a newly emerged pathogen.

In other instances, diseases that were no longer causing widespread infection suddenly began to infect an ever-larger number of individuals. These are referred to as "re-emerging"

TABLE **17-4** Emerging pathogens recognized since 1973

Year	Pathogen	Disease
1973	Rotavirus	Major cause of infantile diarrhea globally
1974	Hepatitis C	Non-A, non-B hepatitis commonly transmitted via transfusions
1976	*Cryptosporidium parvum*	Acute chronic diarrhea
1977	Ebola virus	Ebola haemorrhagic fever
	Legionella pneumophilia	Legionnaires' disease
	Hantavirus	Haemorrhagic fever with renal syndrome
	Campylobacter jejuni	Enteric diseases distributed globally
1980	Human T-lymphotrophic virus I (HTLV-1)	T-cell lymphoma
1981	Toxin-producing strains of *Staphylococcus aureus*	Toxic shock syndrome
1982	*Escherichia coli* 0157:H7	Haemorrhagic colitis
	HTLV-II	Hairy cell leukemia
	Borrelia burgdorferi	Lyme disease
1983	HIV	AIDS
	Helicobacter pylori	Peptic ulcers
1988	Hepatitis E	Enteric non-A, non-B hepatitis
1990	Guanarito virus	Venezuelan haemorrhagic fever
1991	*Encephalitozzon hellem*	Conjunctivitis, disseminated disease
1992	*Vibrio cholerae* 0139	New strain of epidemic cholera
	Bartonella henselae	Cat scratch disease
1994	Sabia virus	Brazilian haemorrhagic fever
1995	Human herpes virus-8	Associated with Kaposi sarcoma in AIDS patients
1996	TSE causing agent	New variant of Creutzfeldt-Jakob disease (mad cow disease)
1997	Influenza A subtype H5N1	Avian influenza
1999	Influenza A subtype H9N2	New strain of human influenza
	Nipah virus	Encephalitis
	West Nile virus	Encephalitis

SOURCE: Adapted from M. F. Good et. al., 1988, *Annual Review of Immunology,* Vol. 6.

infectious diseases. The re-emergence of these diseases should not be surprising if we consider that bacteria can adapt to living in almost any environment. If they can adapt to living at the high temperatures of the thermal vents deep within the oceans, it is not difficult to accept that they can evolve to evade antimicrobial drugs. (An additional risk from intentionally disseminated diseases is discussed in the Clinical Focus.)

Tuberculosis is a well-known re-emerging disease. Fifteen years ago, public health officials were convinced that tuberculosis would soon disappear as a major health consideration in the United States. Then, because of a number of events, including the AIDS epidemic, thousands of infected individuals developed TB strains resistant to the conventional battery of antibiotics. These individuals then passed on the newly emerged, antibiotic-resistant strains of *M. tuberculosis* to others. While the rate of infection with *M. tuberculosis* in the United States increased sharply during the early part of the 1990s, by 1995 the incidence had begun to decline again. However, the worldwide incidence of the disease is still increasing, and the World Health Organization predicts that, between 1998 and 2020, one billion more people will become infected and over 70 million will die from this disease if preventive measures are not adopted.

Another re-emerging disease is diphtheria. This disease was almost non-existent throughout Europe in recent years because of vaccination; in 1994, however, scattered cases were reported in some of the republics of the former Soviet Union. By 1995, there were over 50,000 cases reported in the same region, and thousands died from diphtheria infection. The social upheaval and instability that came with the breakup of the Soviet Union was almost certainly a major factor in the re-emergence of this disease, because of the resultant lapses in public health measures—perhaps most important was the loss of immunization programs. Since 1995, immunization programs have been re-established and the trend has reversed, with only 13,687 cases of diphtheria reported in Russian republics in 1996, 6932 in 1998, and 1573 in 2000.

Other diseases have appeared seemingly from nowhere and, as far as we know, are new pathogens. These include

CLINICAL FOCUS

The Threat of Infection from Potential Agents of Bioterrorism

The use of human pathogens as weapons has a long history. Lord Jeffery Amherst used smallpox against native American populations before the Revolutionary War, and there are reports of attempts to spread plague and anthrax in both the distant and recent past. A few years ago, members of a dissident cult in Oregon introduced salmonella into the salad bars of several restaurants in an attempt cause sickness and death. The more recent discovery of anthrax spores mailed to congressmen and news offices accelerates our interest in possible agents of bioterrorism.

Pathogens and toxins with potential for use as weapons are called "select agents" and include bacteria, bacterial toxins, and certain viruses (see table). The threat from such agents depends on both the severity of the disease it causes and the ease with which it can be disseminated. For example, Ebola virus causes a fulminating hemorraghic disease, but spread of the virus requires direct contact with infected fluids. More worrisome are pathogens that can be spread by aerosol contact, such as anthrax, and toxins that can be added to food or water supplies, such as botulinum toxin.

Category A agents of bioterrorism
Anthrax (*Bacillus anthracis*)
Botulism (*Clostridium botulinum* toxin)
Plague (*Yersinia pestis*)
Smallpox (*Variola major*)
Tularemia (*Francisella tularensis*)
Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg] and arenaviruses [e.g., Lassa, Machupo])

It is ironic that one of the most feared bioterrorism agents is smallpox, the target of the first vaccine. Smallpox is caused by the virus *Variola major;* 30% or more of those infected with this virus die within a month of exposure. Survivors may be horribly scarred. Smallpox can spread rapidly, even before symptoms are visible. As described in Chapter 1, the vaccine for smallpox is a virus (*Vaccinia*) related to variola, which in most cases causes a localized pustule that resolves within 3 weeks. Smallpox disappeared as a consequence of widespread vaccination—the last reported case of natural infection was in 1977. As the disease was eradicated, vaccination was discontinued. In the United States, vaccination ceased in 1972. Production of the vaccine ceased and the remaining doses were put into storage.

Reasons for discontinuing smallpox vaccination include side effects that affect approximately 40 individuals per million vaccinees. These can be life threatening and take the form of encephalitis or disseminated skin infection. In addition, recently vaccinated individuals can spread the virus to others, especially those with compromised immunity. The occasional negative reactions to vaccinia can be treated by the administration of immunoglobulin isolated from sera of persons previously vaccinated, but this so-called Vaccina IG, or VIG, is no longer produced and little remains available. Facing the threat of smallpox as a bioterrorism agent means that vaccination must be reconsidered. It is unlikely that the vaccine produced today will be the same one used earlier. Vaccine was produced by infection of the scarified skin of calves and virus was collected by scraping the infected area. Most likely a new vaccine candidate will be produced under controlled conditions in a tissue-cultured cell line that is certified free of any contaminating viruses. Furthermore, the actual virus used may be a more highly attenuated form of vaccinia. Stocks of VIG must be replenished before a mass vaccination effort is begun.

Most of the viruses on the select agent list are not easy to disseminate. Agents of bioterrorism prepared in a form that allows easy dispersal are referred to as *weaponized.* While nightmare scenarios include customized viral agents engineered in the laboratory, the more likely weaponized pathogens are bacteria. An accidental release of anthrax (*Bacillus anthracis*) in Sverdlovsk in the former Soviet Union infected 79 persons, of whom 68 died, pointing to the deadly potential of this organism. In late 2001, mail containing anthrax (see the accompanying figure) infected a number of persons in multiple postal centers as the letters progressed to their destinations, giving a glimpse of how widely and rapidly a bioweapon might be spread through modern infrastructure.

Bacillus anthracis is a common veterinary pathogen, and like smallpox was the subject of early vaccine efforts, in this case by Louis Pasteur. Human infection was found mainly in those working with hair or hides from animals, especially goats. Infection occurs via three different routes:

- Inhalation causes severe flu-like illness with high mortality unless diagnosed and treated immediately

such pathogens as the widely publicized Ebola virus and *Legionella pneumophilia,* the bacterial causative agent for Legionnaires' disease. Ebola was first recognized after an outbreak in Africa, in 1976. By 1977, the virus that causes this disease had been isolated and classified as a filovirus, a type of RNA virus that includes Marburg virus, a close relative of Ebola. Ebola causes a particularly severe haemorrhagic fever that kills more than 50% of those infected. Because of the

with antibiotics such as penicillin, doxycycline or ciprofloxacin.

- Cutaneous exposure results in skin lesions with characteristic black deep eschar. Cutaneous anthrax has a 20% mortality if untreated, but usually responds to antibiotics.
- Gastrointestinal exposure results in ulcers in the ileum or cecum, bloody diarrhea, and sepsis, and is nearly always fatal because of difficulty in diagnosis.

B. anthracis is particularly deadly because the bacillus forms spores that are quite stable to heat, dryness, sunlight, and other factors that normally limit pathogen viability. It is relatively simple to induce spore formation, and it is spores that are used as bioweapons. Primate studies suggest that inhalation of 2500 to 55,000 spores will cause fatal disease, although the number is controversial. Victims may have flu-like symptoms; a chest x-ray will reveal a characteristic widening of the mediastinum, and blood smears will show gram-positive bacilli. Since prompt diagnosis and treatment is required for survival it is essential that medical personnel recognize the disease.

A vaccine has been developed for anthrax, but its use has been limited to the military. The present preparation is a filtrate from cultures of a non-spore-forming strain of *B. anthracis.* Newly proposed vaccines take advantage of the information gained from basic studies of the mechanism used by the organism to infect target cells, as well as our understanding of the structure and function of anthrax-derived proteins. The major protein involved in infection is the so-called protective antigen, or PA, which pairs with either edema factor (EF) or lethal factor (LF) to cause productive infection. Antibodies that target the binding site on PA for either LF or EF are being developed as the next generation of vaccines against anthrax.

The threat from select agents of bioterrorism, like that from emerging diseases, is being addressed by careful attention to unusual infection events, and by increased study of agents that lend themselves to weaponization. Research to determine the efficacy of various treatments and the windows of immunity that result from administration of antitoxins have risen to top priority in the U.S. following the events of September 11, 2001.

Letters to congressmen and news agencies that contained anthrax spores.
Courtesy of the Federal Bureau of Investigation.

severity of disease and the rapid progression to death after the initial appearance of symptoms, this virus has received a great deal of attention. However, while the risk of death is very high if you are infected with Ebola, it is fairly easy to control the spread of the virus. Through isolation of infected individuals, hospital workers and medical personnel can be protected. In such ways, the spread of Ebola virus has been contained during the two most recent outbreaks.

Another emerging disease recently described is Legionnaires' disease, a virulent pneumonia first reported in 221 individuals who had attended an American Legion convention in Philadelphia in 1976. Of the 221 afflicted, 34 died from the infection. The organism causing the disease was not known, but further investigation led to the identification of a bacterium that was named *Legionella pneumophilia.* This bacterium proliferates in cool, damp areas and can be found in the condensing units of large commercial air-conditioning systems. The air-conditioning system can produce an aerosol that contains the bacteria, thus spreading the infection throughout the area served by the unit. This was determined to be the source of the bacteria at the 1976 convention in Philadelphia. Because the hazard of such aerosols is now recognized, improved design of air-conditioning and plumbing systems has greatly reduced the incidence of the disease.

In 1999, a new virus emerged in the Western Hemisphere. West Nile virus was first isolated in Uganda in 1937, but until recently it was not found outside Africa and western Asia. In 1999, West Nile virus was found in the New York City metropolitan area and by summer 2002, incidence of West Nile virus was reported in all but a few states in the Northwest, indicating a rapid spread of this virus in a short period of time. West Nile virus belongs to a group of viruses known as flaviruses, a group of viruses spread by insects, usually mosquitoes. The most common reservoir of the virus is birds. Crows are particularly sensitive to infection by this virus. Mosquitoes bite an infected bird and, most commonly, the virus-infected mosquito passes the virus to another bird. However, on occasion, the mosquito bites a human, infecting that individual with the virus. Since West Nile is not contagious between humans, it cannot be spread among human populations. In all but a small proportion of humans, West Nile infection does not cause disease. Only in individuals with compromised immune function is the virus a health hazard. Because this virus can cross the blood–brain barrier in compromised individuals, it can cause life threatening encephalitis or meningitis and this is the usual cause of death. Between 1999 and 2001, West Nile caused 18 deaths and sickened 131 others. By September 6, 2002, 954 cases of West Nile had been reported to CDC and 43 people had died in the year 2002. These statistics indicate that West Nile is spreading and is a virus to monitor carefully. Current public health control mechanisms include education of the public regarding mosquito control.

Why are these new diseases emerging and others re-emerging? One reason suggested by public-health officials is the crowding of the world's poorest populations into very small places within huge cities. Another factor is the great increase in international travel; it is now easy to traverse the globe in a very short time, making it possible for an individual to become infected on one continent and then spread the disease to another continent tens of thousands of miles distant. Other features of modern life that may contribute include mass distribution of food, which exposes large populations to potentially contaminated food, and unhygienic food preparation. The World Health Organization and the U. S. Center for Disease Control both actively monitor new infections and work together closely to detect and identify new infectious agents and to provide up-to-date information for travelers to parts of the world where such agents may pose a risk.

SUMMARY

- Innate immune responses form the initial defense against pathogens. These include physical barriers, such as skin, as well as the nonspecific production of complement components and certain cytokines in response to infection by various pathogens.
- The immune response to viral infections involves both humoral and cell-mediated components. Antibody to a viral receptor can block viral infections of host cells. However, a number of viruses, including influenza, are able to mutate their receptor molecules and thus evade the humoral antibody response (see Figure 17-6). Once a viral infection has been established, cell-mediated immunity appears to be more important than humoral.
- The immune response to extracellular bacterial infections is generally mediated by antibody. Antibody can induce localized production of immune effector molecules of the complement system, thus facilitating development of an inflammatory response. Antibody can also activate complement-mediated lysis of the bacterium, neutralize toxins, and serve as an opsonin to increase phagocytosis. Some bacteria secrete protease enzymes that cleave IgA dimers, thus reducing the effectiveness of IgA in the mucous secretions. Other bacteria escape phagocytosis by producing surface capsules or proteins that inhibit adherence to phagocytes, by secreting toxins that kill phagocytes, or by their ability to survive within phagocytes. Host defense against intracellular bacteria depends largely on $CD4^+$ T-cell–mediated responses.
- Both humoral and cell-mediated immune responses have been implicated in immunity to protozoan infections. In general, humoral antibody is effective against blood-borne stages of the protozoan life-cycle, but once protozoans have infected host cells, cell-mediated immunity is necessary. Protozoans escape the immune response through several mechanisms. Some—notably, *Trypanosoma brucei*—are covered by a glycoprotein coat that is constantly changed by a genetic-switch mechanism (see Figure 17-12). Others (including *Plasmodium*, the causative agent of malaria) slough off their glycoprotein coat after antibody has bound to it.
- Helminths are large parasites that normally do not multiply within cells. Because few of these organisms are carried by an affected individual, immune-system exposure to helminths is limited; consequently, only a low level of immunity is induced. Although helminths generally are attacked by antibody-mediated defenses, these may be ineffective. A cell-mediated response by $CD4^+$ T cells plays a critical role in the response to *Schistosoma.*

- Emerging and re-emerging pathogens include some that are newly described and others that had been thought to be controlled by public-health practices. Factors leading to the emergence of such pathogens include increased travel and intense crowding of some populations.

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USEFUL WEB SITES

http://www.cdc.gov/ncidod/

National Center for Infectious Diseases home page—a superb site for monitoring emerging diseases. This is a subdivision of the Centers for Disease Control (CDC), and links to CDC are found at this site.

http://www.niaid.nih.gov/

National Institute of Allergy and Infectious Diseases home page—NIAID is the NIH institute that sponsors research in infectious diseases, and its Web site provides a number of links to other relevant sites.

http://www.who.int/

World Health Organization home page—the international organization that monitors infectious diseases worldwide.
http://www.hopkins-biodefense.org/
The Johns Hopkins University Center for Civilian Biodefense Strategies; in particular, the link entitled "Dark Winter: A bioterrorism exercise" is excellent.

Study Questions

CLINICAL FOCUS QUESTION VIG is used to treat individuals who display complications following administration of the smallpox vaccine. Where is VIG obtained and why is it frequently an effective treatment?

1. The effect of the MHC on the immune response to peptides of the influenza virus nucleoprotein was studied in H-2^b mice that had been previously immunized with live influenza virions. The CTL activity of primed lymphocytes was determined by in vitro CML assays using H-2^k fibroblasts as target cells. The target cells had been transfected with different H-2^b class I MHC genes and were infected either with live influenza or incubated with nucleoprotein synthetic peptides. The results of these assays are shown in the table below.

   a. Why was there no killing of the target cells in system A even though the target cells were infected with live influenza?

   b. Why was a CTL response generated to the nucleoprotein in system C, even though it is an internal viral protein?

c. Why was there a good CTL response in system C to peptide 365–380, whereas there was no response in system D to peptide 50–63?

d. If you were going to develop a synthetic peptide vaccine for influenza in humans, how would these results obtained in mice influence your design of a vaccine?

Target cell (H-2^k fibroblast)	Test antigen	CTL activity of influenza-primed H-2^b lymphocytes (% lysis)
(A) Untransfected	Live influenza	0
(B) Transfected with class I D^b	Live influenza	60
(C) Transfected with class I D^b	Nucleoprotein peptide 365–380	50
(D) Transfected with class I D^b	Nucleoprotein peptide 50–63	2
(E) Transfected with class I K^b	Nucleoprotein peptide 365–380	0.5
(F) Transfected with class I K^b	Nucleoprotein peptide 50–63	1

2. Describe the nonspecific defenses that operate when a disease-producing microorganism first enters the body.

3. Describe the various specific defense mechanisms that the immune system employs to combat various pathogens.

4. What is the role of the humoral response in immunity to influenza?

5. Describe the unique mechanisms each of the following pathogens has for escaping the immune response: (a) African trypanosomes, (b) *Plasmodium* species, and (c) influenza virus.

6. M. F. Good and coworkers analyzed the effect of MHC haplotype on the antibody response to a malarial circumsporozoite (CS) peptide antigen in several recombinant congenic mouse strains. Their results are shown in the table below.

a. Based on the results of this study, which MHC molecule(s) serve(s) as restriction element(s) for this peptide antigen?

b. Since antigen recognition by B cells is not MHC restricted, why is the humoral antibody response influenced by the MHC haplotype?

Strain	H-2 alleles					Antibody response to CS peptide
	K	IA	IE	S	D	
B10.BR	*k*	*k*	*k*	*k*	*k*	<1
B10.A (4R)	*k*	*k*	*b*	*b*	*b*	<1
B10.HTT	*s*	*s*	*k*	*k*	*d*	<1
B10.A (5R)	*b*	*b*	*k*	*d*	*d*	67
B10	*b*	*b*	*b*	*b*	*b*	73
B10.MBR	*b*	*k*	*k*	*k*	*q*	<1

SOURCE: Adapted from M. F. Good et al., 1988, *Annu. Rev. Immunol.* 6:633.

7. Fill in the blanks in the following statements.

a. The current vaccine for tuberculosis consists of an attenuated strain of *M. bovis* called _____.

b. Variation in influenza surface proteins is generated by _____ and _____.

c. Variation in pilin, which is expressed by many gram-negative bacteria, is generated by the process of _____.

d. The mycobacteria causing tuberculosis are walled off in granulomatous lesions called _____, which contain a small number of _____ and many _____.

e. The diphtheria vaccine is a formaldehyde-treated preparation of the exotoxin, called a _____.

f. A major contribution to nonspecific host defense against viruses is provided by _____and _____.

g. The primary host defense against viral and bacterial attachment to epithelial surfaces is _____.

h. Two cytokines of particular importance in the response to infection with *M. tuberculosis* are _____, which stimulates development of T_H1 cells, and _____, which promotes activation of macrophages.

8. Discuss the factors that contribute to the emergence of new pathogens or the re-emergence of pathogens previously thought to be controlled in human populations.