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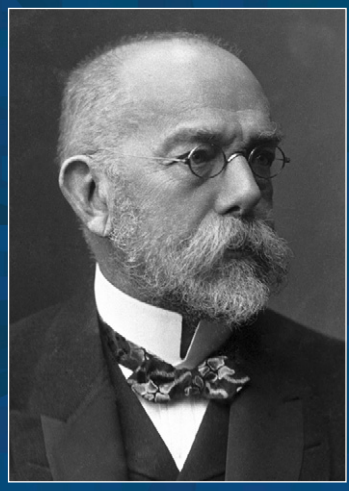
# 22

# Immunity to Pathogens

Robert Koch 1843–1910



*Mycobacterium tuberculosis*
Nobel Prize 1905



**CHAPTER 22**


A. OVERVIEW
B. IMMUNITY TO EXTRACELLULAR BACTERIA
C. IMMUNITY TO INTRACELLULAR BACTERIA
D. IMMUNITY TO VIRUSES
E. IMMUNITY TO PARASITES
F. IMMUNITY TO FUNGI
G. THE MYSTERIOUS PRIONS

*"Never will the doctrine of spontaneous generation recover from the mortal blow of this simple experiment. No, there is now no circumstance known in which it can be affirmed that microscopic beings came into the world without germs, without parents similar to themselves"*—Louis Pasteur

In the first part of this book, we examined each element of innate and adaptive immune responses essentially in isolation, detailing how each developed and functioned. In the second part, we examine the immune response holistically, describing its role in the defense of health against assault from without and within. We discuss what happens when an immune response is unwanted, and the clinical consequences of abnormal or absent immune responses. In this chapter, we focus on defense against pathogens.

Infectious diseases were the leading cause of natural deaths until the early part of the 20th century. In 1900, the three most common causes of death in the United States were pneumonia, tuberculosis, and diarrhea/enteritis. These infectious diseases, along with diphtheria, were responsible for one-third of all deaths. However, due to the late 19th century discovery that specific microorganisms were the cause of such lethal diseases, public health initiatives soon led to dramatic improvements in infectious disease control. Better sanitation and hygiene practices were introduced, including proper sewage disposal, water treatment, and public education about the virtues of handwashing. The discovery of the first antibiotic—penicillin—in 1928 led to its development as a medical treatment beginning in the 1940s. Since that time, many other antibiotics have come into common use, providing cures in countless cases of otherwise fatal bacterial infections. In addition, in North America, Europe, and much of the Pacific Rim, the development of vaccines and effective public vaccination strategies have all but eliminated potentially lethal diseases such as diphtheria, measles, mumps, and rubella that were once endemic in childhood. The success of these public health measures can hardly be overstated: since 1900, human average life expectancy has increased by 30 years. There has been a corresponding drop in infant and child mortality rates in developed countries such that the proportion of annual deaths in this age group due to infectious disease has fallen to less than 2% from over 30%. Despite these gains, of the approximately 57 million human deaths that occur annually around the world today, about 25% are still due to infectious diseases, considerably more deaths than can be attributed to cancer, cardiovascular disease, or trauma. While new types of antibiotics and vaccines have provided great boons in eradicating the threat posed by some deadly pathogens, others continue to defy all our attempts to control them.

In the following sections, we cover how all the elements detailed in Chapters 1–20 combine to eliminate five major classes of human pathogens. The common physical barriers and innate defense mechanisms encountered by all pathogens are discussed first. We then describe, for each class of pathogen in turn, the immune responses commonly mounted against that class of pathogen, followed by a discussion of the evasion strategies employed by that class to stave off destruction. A short discussion of *prions*, a new category of pathogen that involves infectious proteins, appears at the end of this chapter. Vaccination, which primes a host's immune response to better combat infection, is addressed in Chapter 23.

## A. Overview

### I. WHAT IS A "PATHOGEN"?

The life objective of any organism is to reproduce, and a pathogen is an organism that can cause disease in its host while attempting to achieve this goal. There are five major types of pathogens: *extracellular bacteria*, *intracellular bacteria*, *viruses*, *parasites*, and *fungi*. Bacteria are microscopic, single-celled organisms that are considered prokaryotic because they do not have the "true" nucleus found in eukaryotes. The prokaryotic nucleus lacks a nuclear membrane and the genetic material of these organisms is usually contained in a single linear chromosome. Extracellular bacteria do not have to enter host cells to reproduce, while intracellular bacteria do. At this point, the reader should take a moment to familiarize him- or herself

## Box 22-1. Classification of bacteria

Bacteria have been traditionally classified on the basis of their shapes and groupings, their use of oxygen, and their gram stain status. Despite the advent of DNA technology, these terms of reference remain entrenched in the microbiological research community. For our discussions of bacteria in the context of immunology, we have divided the bacteria into the "extracellular" and "intracellular" categories, depending on where they live in the body in relation to host cells. Both these categories contain members of each of the groups described here.

### Shapes and Groupings

Bacterial cells come in various shapes and groupings that are associated with specific nomenclature. The most common bacterial shapes are rod-like, which are the *bacilli* ("little staffs"; singular is "bacillus"); spherical or ovoid, which are the *cocci* ("berries"; singular is "coccus"); and *comma forms* and *spirals*. Bacteria may also be star-shaped or square, although these shapes are less common. Cocci are commonly grouped in pairs (*diplo*), chains (*strepto*) or clusters (*staphylo*), giving rise to diplococci, streptococci, and staphylococci. Bacilli are found in pairs and chains, giving rise to diplobacilli and streptobacilli. Spirals are usually found singly, but may occur as *spirilla* (rigid helix) or *spirochetes* ("flexible helix"). *Vibrios* are comma-shaped bacteria.

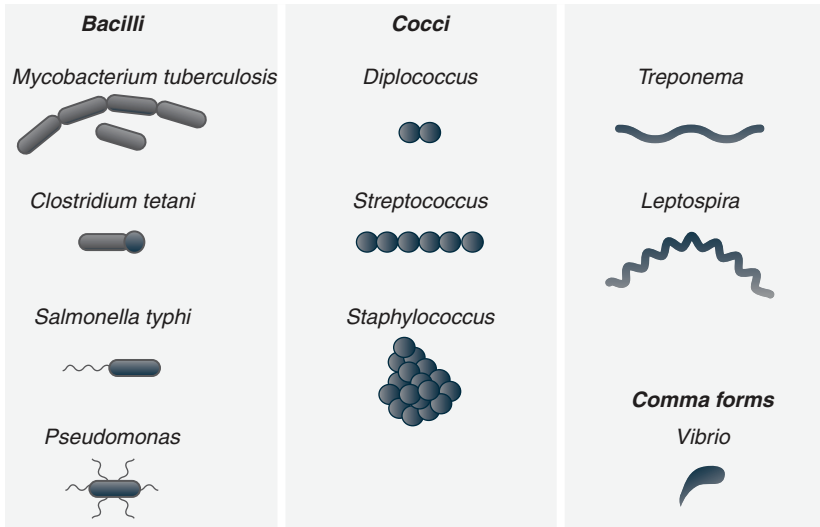
### Use of Oxygen

Microbes in general and bacteria in particular are often described with respect to their use of oxygen. Those that can use oxygen are called *aerobes*, while those that do not are called *anaerobes*. A microbe that uses oxygen when available but can live anaerobically in the absence of oxygen is said to be a *facultative aerobe*. Those microbes that must have oxygen to survive are called *obligate aerobes*, while those that can grow only in the complete absence of molecular oxygen are called *obligate anaerobes*.

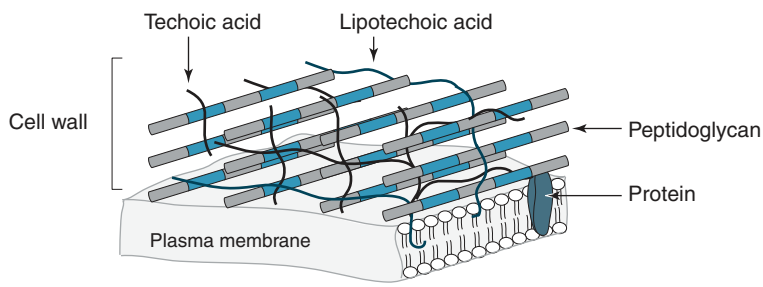
### Gram Stain Status

Bacteria are also classified into two groups according to how they appear when stained in the laboratory using the multi-step gram stain procedure developed in 1884 by Hans Christian Gram. One group of bacteria has cell walls with many layers of peptidoglycan interwoven with both lipotechoic and techoic acid. These relatively thick cell walls retain the dark purple color imposed by the initial step

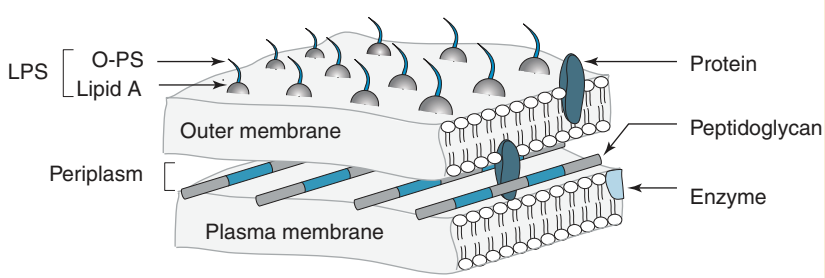
**A. Bacterial Shapes and Cell Wall Components**



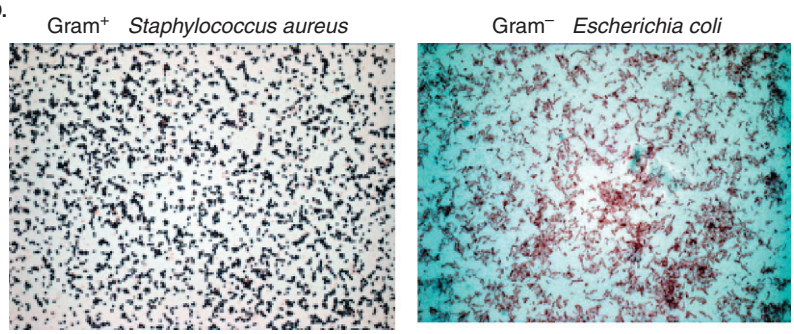

**B. Gram-Positive Cell Wall**




**C. Gram-Negative Cell Wall**




**D.**




*Continued*

**Box 22-1. Classification of bacteria—*cont'd***

of the staining procedure despite undergoing a later decolorizing step and counterstaining with the red dye safranin. Bacteria staining purple in this fashion are called "gram-positive" bacteria. The other group, called "gram-negative" bacteria, have a cell wall with a thin peptidoglycan layer that loses its integrity during the staining procedure, allowing decolorization to occur. The purple color vanishes, allowing the cells to stain red with safranin. As well as peptidoglycan, the gram-negative cell wall has an outer plasma membrane containing lipopolysaccharide macromolecules composed of Lipid A (conserved among all gram-negative species) and an O-linked polysaccharide (O-PS; variable among gram-negative species). Functionally, the O-linked polysaccharide confers virulence (the ability to infect host cells) on the bacterium. It is the Lipid A moiety that functions as an endotoxin capable of inducing the endotoxic shock described in Box 17-2.

Gram-negative and gram-positive bacteria can also be distinguished by their differential susceptibility to physical assaults as well as various enzymes and drugs. For example, gram-positive bacteria resist dry conditions better than gram-negative bacteria and cannot be crushed as readily. Gram-positive bacteria are more resistant to lysozyme digestion and killing by streptomycin and tetracycline, but are highly susceptible to penicillin, anionic detergents, and basic dyes. In contrast, gram-negative bacteria resist lysozyme digestion and killing by penicillin but dry out easily, are inhibited by basic dyes, and are susceptible to killing by tetracycline.

Panel B adapted from "Microbiology, An Introduction," 6th and 7th edns. (2002, 2004) Gerard J. Tortora, Berdell R. Funke, Christine L. Case, eds. Published by Benjamin Cummings, San Francisco. Panel D courtesy of Dr. Tony Mazzulli, Mount Sinai Hospital, Toronto.

with the common terms and concepts used in classifying or discussing bacteria (Box 22-1). Viruses are not considered to be either prokaryotic or eukaryotic. Viruses are acellular particles consisting of a protein coat (called a *capsid*) encasing a genome of either DNA or RNA. Due to their small size, viruses can only be observed with an electron microscope. To propagate, a virus must enter a host cell possessing protein synthesis machinery that the virus can exploit. Parasites and fungi are eukaryotic organisms that possess several chromosomes contained in a membrane-bounded nucleus. Parasites all share the characteristic of taking advantage of and being dependent on a host organism for both habitat and nutrition at some point in their life cycles, usually damaging the host but not killing it. The major classes of parasites are the protozoa, which are single-celled organisms, and the helminth worms, which are multicellular. While some parasites may be only a few micrometers in size, many are significantly larger, with some worms reaching several meters in length. Fungi are eukaryotic organisms that can exist comfortably outside a host but that will invade and colonize a host if given the opportunity. Fungi may be single-celled, as in the case of yeast, or multicellular, as in bread molds. Prions are curious entities that are infectious but appear to be made entirely of protein and lack any type of nucleic acid. Prions cause disease by altering normal proteins in the brain of the infected host.

## II. WHAT IS "DISEASE"?

Disease is not the same as infection. Infection is said to have occurred when an organism successfully avoids innate defense mechanisms and stably colonizes a niche in the body. To establish an infection, the invader must first penetrate the anatomic and physiological barriers that guard the skin and mucosal surfaces of the host. Secondly, the organism must be able to survive in the host cellular milieu long enough to reproduce. This replication may or may not cause visible, clinical damage to the host tissues, symptoms that we call "disease." It should be noted that sometimes the products released by a pathogen, such as bacterial toxins, are sufficient to cause disease even in the absence of widespread pathogen colonization. An "emerging infectious disease" is a disorder that has come to light only within the past couple of decades, or is changing in character such that it has an increased impact on the global population. Table 22-1 contains short descriptions of several emerging infectious diseases.

In addition to direct destruction by pathogen actions, host tissues are often injured by collateral damage inflicted by the immune response itself as it strives to destroy the pathogen. This damage results in *immunopathic* disease symptoms. CTLs may directly induce the lysis of bystander host cells via cytotoxic cytokine secretion, or phagocytes may release antimicrobial factors (like $H_2O_2$ or free radicals) that are also toxic to host cells. A very dramatic example of immunopathic damage occurs in mice infected in the brain with *lymphocytic choriomeningitis virus* (LCMV). Elsewhere in the body, this virus is quite harmless to the animals. In the brain, however, the CTL response to the virus results in neuron cytolysis that kills the mice. The endotoxic shock triggered by the LPS of gram-negative bacteria (refer to Box 17-2) is another example of immunopathic damage. Several other examples are given in Table 22-2.

*Virulence* is the ability to invade host tissues and cause disease in the host; the more virulent a pathogen is, the better able it is to resist the immune system. Many factors affect virulence: how quickly the pathogen can access a host cell and/or replicate, whether the pathogen can express molecules that damage the host or dampen its immune response, and whether the host MHC and PRR molecules can recognize the pathogen's processed peptides and PAMPs, respectively. If the immune system is able to eliminate the organism before it can successfully propagate, the infection is said to have been *abortive*. If, however, the pathogen multiplies despite the actions of the immune system, the infection is *productive*. Whether an infection is abortive or productive depends on the initial dose of the pathogen accessing the host, the virulence of the organism, and the strength and rapidity of the immune responses mounted to

Table 22-1 **Emerging Infectious Diseases**

| Disease | Pathogen | Recent Outbreak | Description |
|---|---|---|---|
| Cryptosporidiosis | *Cryptosporidium* (protozoan) | 1993 | Diarrheal illness transmitted by contaminated water |
| Pulmonary syndrome | Hantavirus | 1993 | Fever, cough, rapid respiratory failure; carried by deer mice |
| Hemorrhagic fever | Ebola virus | 1995 | Bleeding from all orifices of body, followed by clotting in blood vessels |
| Necrotizing fasciitis (flesh-eating disease) | Group A *Streptococcus* | 1995 | Rapid and progressive loss of tissue layers; can require amputation of infected limb |
| West Nile encephalitis | West Nile virus | 1999 | Ranges from flu-like illness to potentially fatal brain infection; mosquito-borne |
| Hamburger disease | *E. coli* 0157:H7 | 2001 | Bloody diarrhea, severe dehydration due to consumption of uncooked meat or contaminated water |
| Severe acute respiratory syndrome (SARS) | Coronavirus | 2003 | Respiratory disease resembling atypical pneumonia; can be fatal |

combat the invasion. The latter will be determined in part by whether the host has been previously exposed to the pathogen in question. In the last phase of a productive infection, the progeny of the pathogen escape the original host and travel to new ones.

An ongoing struggle or "horse race" is thus established between a pathogen and the immune system: the pathogen tries to replicate and expand its niche, and the immune system tries to eliminate the pathogen, or at least confine it.

Table 22-2 **Examples of Immunopathic Disease Symptoms**

| Disease Symptom | Immunopathic Cause |
|---|---|
| Endotoxic shock | Flood of pro-inflammatory cytokines released in response to LPS of gram-negative bacteria |
| Flu-associated malaise and fever | Pro-inflammatory cytokines released to fight influenza virus |
| Hepatitis virus-associated liver damage | Fas-mediated apoptosis of hepatocytes |
| HTLV-associated spinal cord damage | Chronic inflammatory response to residual viral antigen |
| LCMV-associated brain pathology | Pro-inflammatory cytokines released to fight LCMV kill neurons in the brain |
| Measles red rash | Cell-mediated immune response against infected cells in skin |
| Tuberculosis | Granuloma formation in the lungs caused by hyperactivated macrophage response to bacteria |
| Ulcers | Cytokines produced by Th1 cells responding to *Helicobacter pylori* infection in the gut |

The interval between the time of infection and the onset of disease is called the *incubation time*, which can vary considerably in length depending on the lifestyle of the pathogen. An infection is said to be *acute* when it causes disease symptoms that appear rapidly but remain for only a short time. A *persistent* infection is one in which the pathogen remains in the host's body for prolonged periods or possibly throughout life. Persistent infections may result in *chronic disease* if symptoms are experienced on an ongoing or recurring basis. In other cases, a persistent pathogen may lurk for an extended time without producing any symptoms, in which case the infection is *latent*. A pathogen may or may not be infectious during latency. A person with a latent infection that can be transmitted unknowingly to others is a *carrier*.

The threat of pathogen attack has prompted hosts to evolve immune responses designed to repel and neutralize the invaders and stop their appropriation of host resources. Naturally, the pathogens, whose evolution occurs much more rapidly than that of their hosts, have responded with the development of various "evasion strategies" designed to outwit or compromise the host's immune response. For example, a host population may be resistant to a certain pathogen because of an effective neutralizing antibody response that prevents the pathogen from gaining a foothold on host cells. However, if the pathogen undergoes a mutation that allows it to make novel use of a host surface protein to enter the cell, the pathogen can escape the humoral response. The host population is once again susceptible to disease.

## III. INNATE DEFENSE AGAINST PATHOGENS

As described in Chapter 20, the first lines of defense encountered by any pathogen are the intact skin and mucosae. The vast majority of microorganisms cannot penetrate the tough

keratin layer protecting the epidermis, and any pathogen arriving on the skin surface has to compete with the commensal flora for space and nutrients. Furthermore, the routine shedding of skin layers often deposes a pathogen before it has a chance to establish a firm foothold. Similarly in the mucosae, pathogens ingested into the gut or inhaled into the respiratory tract are for the most part trapped by mucus and secretory IgA coating the mucosal surfaces. Microbicidal molecules in the body secretions and the low pH and hydrolases in the gut also take their toll. However, should there be a wound in the skin or the mucosae that allows a pathogen access to tissues underlying the epithelium, the consequences can be severe. Some organisms are *opportunistic* pathogens, in that they do not cause disease unless offered an unexpected opportunity by a failure in host defense. *Staphylococcus aureus* is an example of an extracellular bacterium that is not particularly invasive and that normally colonizes the skin surface without harming the host. If inhaled, *S. aureus* is successfully repelled by an intact respiratory epithelial layer. However, should the skin experience a tear or the respiratory epithelium be damaged by trauma or another more invasive pathogen, *S. aureus* can take advantage of the breach and establish a serious infection. When the opportunity arises to invade the respiratory tract, staphylococcal pneumonia can result. Opportunistic invasions are a major problem in hosts immunocompromised either by disease or by design. For example, patients on immunosuppressive therapy following a tissue transplant, or those whose immune systems have been destroyed by HIV, are vulnerable to infection by the opportunistic pathogens *Pneumocystis carinii* and *Candida albicans*.

A small minority of microorganisms are *invasive* pathogens, capable of entering the body even when surface defenses are intact. As we learned in Chapter 20, some viruses (and bacteria, as we shall see later in this chapter) can target the antigen-collecting M cells of the mucosal follicle-associated epithelium (FAE) to effect entry, while other pathogens have the capacity to adhere to host molecules expressed on cutaneous or mucosal epithelial cells. Still other pathogens exploit host surface molecules as receptors, inducing receptor-mediated internalization. Many highly invasive pathogens can be found in the group A *Streptococcus*, bacteria that cause 15,000 severe infections per year in the United States.

Most times, a pathogen that succeeds in penetrating the skin or mucosae either through a wound or via exploitation of host cell receptors meets effective innate defense in the form of subepithelial macrophages and NK cells and intraepithelial γδ T cells (Fig. 22-1). (Table 22-3 contains a list of the abbreviations used in the figures in this chapter.) Molecules on the pathogen surface may be recognized directly by the PRRs of resident macrophages, by the activatory receptors of NK cells, and by the restricted TCRs of γδ T cells and NKT cells. Inflammatory responses are mounted that first draw neutrophils into the site of incursion. Different types of pathogens elicit the production of different classes of chemokines that facilitate the inflammatory response. For example, acute infection by an extracellular gram-positive bacterium such as *Streptococcus pneumoniae* results in the production of predominantly CXC chemokines, particularly IL-8, that summon the neutrophils required to eliminate the bacteria. The lipopolysaccharides of the cell walls present in gram-negative bacteria such as *Escherichia coli* are also powerful inducers of CXC chemokine secretion. In contrast, infection with a bacterial spirochete such as *Borrelia burgdorferi* leads to a more chronic condition eventually requiring action by macrophages and lymphocytes. Infection with this extracellular organism provokes the secretion by activated macrophages of CC (e.g., MCP-1) or C (e.g., lymphotactin) chemokines that attract the required cells.

Inflammatory cells responding to an invading pathogen produce toxic substances such as nitric oxide (NO) and acute phase proteins, and cytotoxic and activatory cytokines such as TNF and IFNγ. Complement components that either have leaked into the tissue from the blood or were produced by cells in the site of attack may be activated by either the alternative or lectin pathways. The pathogen will then be coated in C3b or C3d, enhancing phagocytosis via complement receptors on phagocytes. MAC-mediated destruction of the pathogen may also ensue if a membranous structure is present. Other complement components serve as chemoattractants, drawing more phagocytic cells to the site of attack. After phagocytes have engulfed an invader, molecules such as lactoferrin are introduced into the phagosome to sequester iron atoms, further inhibiting bacterial growth. Despite all these measures, a pathogen may avoid phagocytosis, opsonized or otherwise, and access the blood circulation. *Bacteremia*, *viremia*, and *parasitemia* are terms referring to the presence in the blood of bacteria, viruses, or parasites, respectively. Innate defense in the blood falls to blood monocytes and neutrophils. Should the pathogen reach the liver or the spleen, the macrophages present in these organs attempt to confine the invader. A pathogen tough enough to escape these measures may successfully colonize other organs and cause serious abscesses.

As the innate response proceeds, the pathogen or some of its components interact with the PRRs expressed on macrophages and DCs and are ingested, resulting in activation of these cell types such that they become capable of acting as APCs for the adaptive response. The products of many species of bacteria and protozoa (particularly lipopolysaccharides) are potent activators of DCs, promoting their maturation into fully competent APCs. As well, the DNA of some parasites and bacteria, particularly if it is unmethylated, can activate DCs via binding to TLR9. The DNA of these organisms, but not that of vertebrates, contains stretches of unmethylated cytosine-phosphate-guanosine residues called *CpG motifs*. TLR9 appears to be specific for these motifs. Lipopolysaccharides also induce the upregulation of the B7 molecules, CD40, and MHC class I and II on the DC surface, and promote DC migration to the secondary lymphoid tissues. Cytokine production by DCs, including TNF, IL-12, IL-10, IL-6, and IFNα/β, is induced. The arrival of activated, antigen-bearing macrophages and DCs in the local lymph node

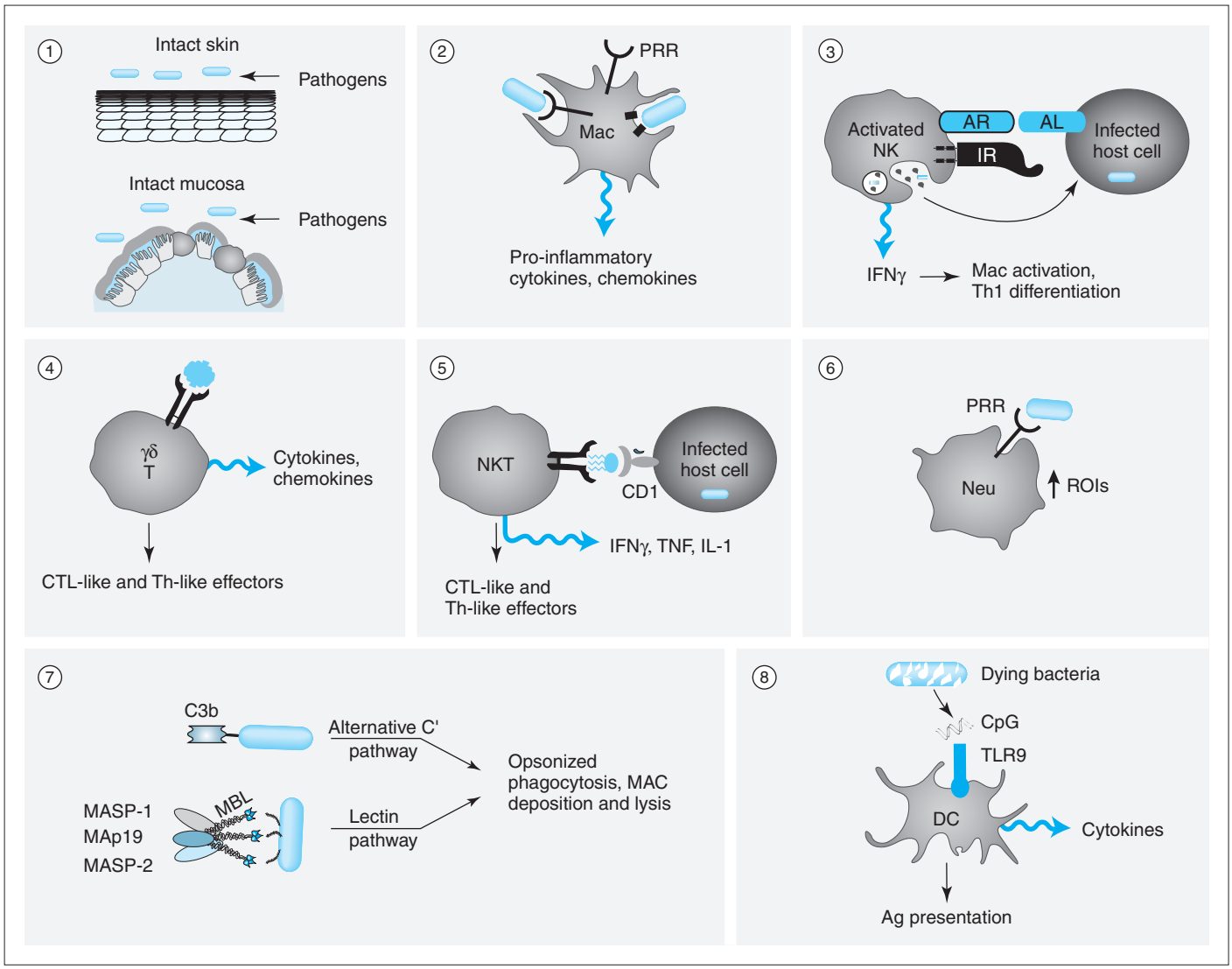



Figure 22-1

**Major Mechanisms of Innate Defense against Pathogens**
(1) Intact skin and mucosa prevent pathogen penetration. (2) Macrophages activated by PRR-mediated recognition of pathogen PAMPs carry out phagocytosis and secrete cytokines. (3) Infected cells that have downregulated MHC class I activate NK cells and are destroyed by natural cytotoxicity. IFNγ secreted by the activated NK cell activates macrophages and supports Th1 differentiation. (4) γδ T cells activated by soluble non-peptide pathogen antigens differentiate into effector cells and secrete cytokines. (5) Infected cells that present pathogen antigens on CD1 activate NKT cells that differentiate into effector cells and secrete cytokines. (6) Neutrophils activated by PRR-mediated recognition of pathogen PAMPs kill phagocytosed pathogens via respiratory burst and increased ROI production. (7) Pathogens that have bound C3b or MBL are destroyed via the alternative or lectin complement activation pathways, respectively. (8) DCs activated by TLR-mediated recognition of pathogen PAMPs take up pathogen debris, increase their presentation of pathogen peptides to T cells, and secrete cytokines. Abbreviations are defined in Table 22-3 on page 648.

stimulates the activation of naive T and B cells. The adaptive immune response then proceeds in its bid to eliminate the pathogen. As is described later, the adaptive response effector mechanisms most effective in countering a pathogen are determined by the invader's lifestyle and mode of replication.

We move now to a discussion of each class of pathogen, the details of the immune response effector mechanisms employed by the host to combat them, and the evasion tactics used by each to escape the immune system.

## B. Immunity to Extracellular Bacteria

### I. WHAT ARE EXTRACELLULAR BACTERIA?

As mentioned previously, extracellular bacteria do not have to enter host cells to replicate. Rather, they occupy spaces topologically outside cells: interstitial regions in connective tissue, the blood circulation, and the lumens of the respiratory, urogenital,

Table 22-3 **Abbreviations Used in Chapter 22 Figures**

| Abbreviation | Definition |
|---|---|
| Act Mac | Activated macrophage |
| Ag | Antigen |
| AL | Activatory ligand (NK cells) |
| AR | Activatory receptor (NK cells) |
| C′ | Complement |
| Cyt c | Cytochrome *c* |
| Eo | Eosinophil |
| FcR | Fc receptor |
| Hyper Mac | Hyperactivated macrophage |
| IR | Inhibitory receptor (NK cells) |
| Mac | Macrophage |
| MAC | Membrane attack complex |
| Mast | Mast cell |
| MBL | Mannose binding lectin |
| Neu | Neutrophil |
| NO | Nitric oxide |
| PC | Plasma cell |
| $PrP^{c}$ | Prion protein, cellular |
| $PrP^{sc}$ | Prion protein, scrapie |
| PRR | Pattern recognition receptor |
| RBC | Red blood cell |
| RNI | Reactive nitrogen intermediates |
| ROI | Reactive oxygen intermediates |
| TLR | Toll-related receptor |
| Uncon CTL | Unconventional CTL |

and gastrointestinal tracts. Extracellular bacteria often secrete proteins that penetrate or enzymatically cleave components of the glycocalyx covering cells of the mucosal epithelium, allowing the invaders access to interior tissue layers. A wide variety of extracellular bacteria gain access to the body via the M cells in the mucosal FAE, while others make use of complement receptors. The exploitation of various host components by pathogens is reviewed briefly in Box 22-2.

The major types of extracellular bacteria causing human disease are represented in the gram-positive cocci (e.g., *Staphylococcus*, *Streptococcus*), gram-positive bacilli (e.g., *Clostridium*, *Corynebacterium*), gram-negative cocci (e.g., meningococcus), and gram-negative bacilli (e.g., *Escherichia*) (Table 22-4). Many of the gram-positive cocci are *pyogenic*, meaning that they induce an acute, immunopathic inflammatory response accompanied by a high fever. In this situation, macrophages responding to the bacterium produce massive amounts of cytokines (particularly IL-1) that spill into the bloodstream and travel to the brain. The hypothalamus of the brain makes prostaglandins in response to these cytokines, and the prostaglandins act on the pituitary gland and influence it to raise the body's temperature.

### i) Characteristics of Selected Species of Extracellular Bacteria

The most common extracellular bacteria causing acute human infections are species of *Streptococcus* and *Staphylococcus*. For example, *Streptococcus pneumoniae* (also known in short-hand as pneumococcus) infection can lead to pneumonia, while group

Box 22-2. **Pathogen entry: exploitation of host components**

Many pathogens have evolved devious means by which to circumvent the innate defenses of the host's body. Among the most popular devices are use of the M cells of the FAE and the subversion of complement receptors and RCA proteins.

#### M Cells

The reader will recall from Chapter 20 that the relatively rare M cells of the FAE are key mediators of the antigen sampling necessary for mucosal immunity. Unfortunately, M cells are also inviting portals of entry for a variety of pathogens. The structure of the M cell is such that it lacks the protective glycocalyx of other mucosal epithelial cells, and forms a pocket that extends deep into the underlying lamina propria. Furthermore, the well-developed endocytic apparatus of the M cell is designed to expedite the passage of foreign material through the cell and into the dome underlying the pocket. Invasive species of bacteria and viruses have exploited this transport mechanism as an easy route to cross the otherwise resistant gut epithelium.

Binding to M cells has been demonstrated for (among several other bacterial species) *Salmonella typhimurium*, *Salmonella enterica*, *Yersinia enterocolitica*, and *Vibrio cholerae*, all of which cause enteric disease. (In contrast, the pathogenic strains of *E. coli* that cause similar symptoms attack gut enterocytes.) Only a few specific receptor–ligand pairs have been identified that exclusively facilitate bacterial entry through M cells. For example, the invasin proteins of *Yersinia* bind to β1 integrins on the M cell apical pole. Entry may be mediated more often by glycoproteins expressed on the apical surfaces of the M cells that can bind to particular bacterial adhesins. While some species of invading bacteria (e.g., *Salmonella*) are toxic to the M cells they have invaded, others are not. For example, *V. cholerae* does not cause disease until it reaches the small intestine, where it commences expression of proteins that allow it to adhere to intestinal epithelial cells. Only then does the bacterium produce the cholera toxin that induces the exodus of chloride ions from the cell that leads to severe diarrhea.

*Shigella flexneri* also exploits M cells to cause enteric disease, but this intracellular bacterium accesses these cells by a different mechanism. In animal models, the M cells take up *S. flexneri* by what appears under the electron microscope to be a macropinocytotic event. In some cases, the bacteria cross the M cell without lysing the endocytic vacuole, travel to the deep end of the pocket, and infect intestinal epithelial cells by the basolateral (rather than the apical) pole (see Figure). In other cases, the *Shigella* are taken up by macrophages within the dome. The bacteria induce the apoptosis of these cells, and with this apoptotic death comes the release of more *Shigella* that can invade neighboring epithelial cells, as well as a flood of IL-1β into the local area that induces inflammation. This inflammation contributes to a destabilization of the epithelial cell layer that makes it easier for the *Shigella* to reach

*Continued*

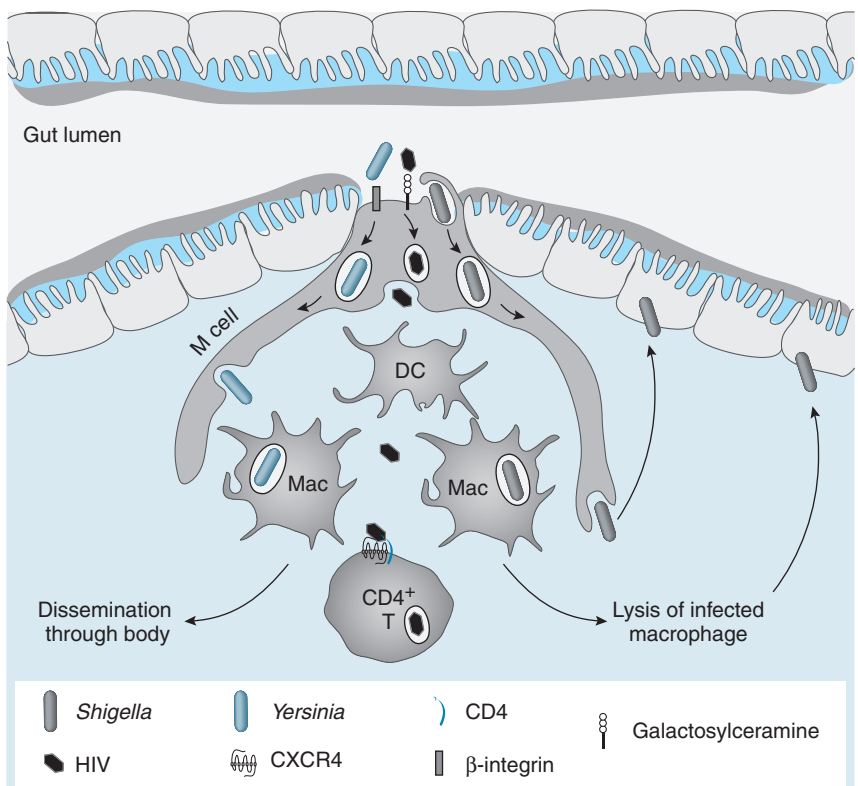
Box 22-2. **Pathogen entry: exploitation of host components–*cont'd***

fresh epithelial cells. *Yersinia* and *Salmonella* also make use of dome macrophages but in a different way, hiding in these cells without harming them while the cells migrate from the FAE throughout the body. The bacteria can then induce apoptosis of the macrophages at a later time and attack epithelial cells in new locations.

Several types of viruses also access the body via M cells. In mice, reovirus entering the gut via the digestive tract is proteolytically modified by host digestive such that the virus is activated. This activation induces the expression of an (unidentified) protein that allows the virus to adhere to M cells in the intestinal PPs and the respiratory tract. The virus uses the pocket of the M cell to access the interior of the follicle and infect FAE cells from the basolateral side. Viral progeny then go on to infect the preferred targets of this virus, the macrophages and neurons in the dome underlying the FAE. Interestingly, DCs are not productively infected by reovirus. Rather, DCs capture viral antigens from apoptotic or necrotic epithelial cells and present them to mucosal T cells to initiate an immune response (refer to Ch.20). Another virus that exploits M cells in a variety of sites in mice is the retrovirus called mouse mammary tumor virus (MMTV). Newborn mice may be infected with MMTV after consuming their infected mother's milk, and the virus then moves from the gut lumen to the M cells of the PPs. The virus spreads from there to the gut epithelial cells, the mesenteric lymph nodes and eventually to all lymphoid organs. In adult mice, MMTV enters the body via the M cells of the nasal or rectal epithelium. In humans, polio virus enters the body orally and passes through the M cells of the PPs on its way to its preferred target cells, the neuronal cells in the lamina propria. HIV is also thought to take advantage of M cells. HIV is frequently transmitted by anal intercourse but the virus cannot penetrate the glycocalyx of healthy rectal epithelial cells. Rather, the virus may enter the body via M cells present in FAE in the rectum, possibly by binding to galactosylceramine expressed on the M cell surface. Studies in rabbits and mice have confirmed that HIV can bind to M cells of the PPs and be transcytosed *in vitro*. Transportation through the M cell into its pocket would give ready access to the T cells, DCs, and macrophages that are this virus's primary targets. More on HIV appears in Chapter 25.

### Complement System Components

Once in the tissues, various pathogens have exploited numerous components of the complement system to either increase their adherence to host cell surfaces or to gain access to the interior of a host cell by receptor-mediated endocytosis. For example, pathogens as varied as HIV, *Legionella pneumophila, Mycobacterium tuberculosis*, and *Leishmania major* all express ligands that allow them to bind to CR1, and several also make use of CR3. CR2 acts as a receptor for the Epstein-Barr virus that mediates infection of epithelial cells and B cells. Many picornaviruses such as the Coxsackie viruses express proteins that allow them to adhere to DAF. Host cell entry is not achieved, however, unless ICAM-1 is present to play an (unknown) coreceptor-like role. Various strains of *Escherichia coli* express different adhesin proteins (*Dr proteins*) that can also adhere to DAF, while *Neisseria gonorrhea*, *Neisseria meningitidis*, and *Helicobacter pylori* express pili that bind to MCP on host cells in target tissues. Some laboratory strains of measles virus and HHV-6 are thought to make similar use of MCP to effect host cell entry.




A streptococci are responsible for the severe throat infections commonly known as "strep throat." *Staphylococcus aureus* is responsible for many minor infections of the skin and mucosae, but also produces toxins causing food poisoning or toxic shock syndrome. A much more serious disease caused by either *Staphylococcus aureus* or group A streptococci alone, or by both in combination, is necrotizing fasciitis, more commonly known as "flesh-eating disease." Necrotizing fasciitis is characterized by the sudden onset of a rapidly spreading inflammatory infection that destroys the fascia, the layers of tissue covering the subcutaneous muscle layers. The skin and subcutaneous tissue become separated from the underlying muscles, and the patient experiences fever, severe pain, and a reddened swelling that spreads extremely quickly under the surface of the affected body part. Affected limbs may have to be amputated. Necrotizing fasciitis most often occurs in adults who have undergone surgery or experienced physical trauma of some sort, or who have compromised immune systems. Thankfully, this

Table 22-4 **Examples of Extracellular Bacteria and the Diseases They Cause**

| Organism | Disease |
|---|---|
| *Bacillus anthracis* | Anthrax |
| *Borrelia burgdorferi* | Lyme disease |
| *Clostridium botulinum* | Botulism |
| *Clostridium tetani* | Tetanus |
| *Corynebacterium diphtheria* | Diphtheria |
| *Escherichia coli* O157:H7 | Hemorrhagic colitis ("hamburger disease") |
| *Helicobacter pylori* | Ulcers |
| *Leptospira* (various) | Leptospirosis |
| *Neisseria gonorrhea* | Gonorrhea |
| *Neisseria meningitidis (Meningococcus)* | Bacterial meningitis |
| *Salmonella enteritidis* | Food poisoning (poultry) |
| *Staphylococcus aureus* | Food poisoning, toxic shock syndrome, eye and skin infections, necrotizing fasciitis |
| *Streptococcus* (Group A) | "Strep throat," necrotizing fasciitis |
| *Streptococcus pneumoniae (Pneumococcus)* | Pneumonia |
| *Treponema pallidum* | Syphilis |
| *Vibrio cholerae* | Cholera |
| *Yersinia enterocolitica* | Severe diarrhea |

disease is rare (incidence of 0.3–0.7 per 100,000 persons per year), but it is fatal in 20–30% of cases.

One of the most feared (but rare) diseases resulting from a gram-positive bacillus infection is botulism, caused by a neurotoxin (see later) produced by *Clostridium botulinum*. *C. botulinum* is an anaerobic bacterium that commonly resides in the form of dormant spores in soil, fresh water, and house dust, and on the surfaces of many foods. Upon introduction into a suitable low oxygen habitat, the bacterium resumes replication and production of the neurotoxin. Unfortunately, such an environment is sometimes provided by home-canned or home-preserved foods that did not receive adequate heat treatment. Within 8–36 hours after consuming food contaminated by even a minuscule amount of *C. botulinum* neurotoxin, the patient experiences blurred or double vision, slurred speech, swallowing and breathing difficulties, and muscle weakness due to paralysis of the muscle caused by the neurotoxin. Recovery may take weeks or months, and 7–8% of patients die.

An example of a pathogenic gram-negative coccus is *Neisseria meningitidis*, a member of the meningococci. *N. meningitidis* causes bacterial meningitis, a severe inflammation of the membranes covering the brain and spinal cord (the meninges). About 10% of the general population carries these bacteria harmlessly in the nose and back of throat. However, in the rare event that these bacteria enter the blood, travel to the brain, and attack the meninges, toxins produced by these organisms induce inflammation that damages the brain. The patient complains of a severe headache, rash, lethargy, and a stiff neck, and avoids looking at bright lights. In some cases, the patient may enter a coma, which can be fatal. For unknown reasons, children under the age of 5 years and college and university students are at particularly high risk for contracting bacterial meningitis. Kissing and the sharing of items such as food and beverage utensils or even musical instrument mouthpieces can transmit the bacterium between individuals.

*E. coli* strain O157:H7 is a gram-negative bacillus that causes hemorrhagic colitis, more commonly known as "hamburger disease." *E. coli* O157:H7 lives harmlessly in the intestines of most food animals, including pigs, chickens, and cattle. During slaughter, the bacterium transfers to the exterior surfaces of the butchered meat. In the case of beef, the grinding of a cut of beef into hamburger distributes the bacterium throughout the meat. If this hamburger not cooked thoroughly and is eaten, the bacterium survives and commences replication in the human gut. The consumption of water contaminated by the manure of farm animals can also cause the disease. The patient experiences hemorrhagic colitis (bloody diarrhea), which may be accompanied by vomiting, abdominal cramps, and fever. A toxin (see later) produced by *E. coli* O157:H7 attacks the endothelial cells lining the body's small blood vessels, allowing the leakage of blood into the tissues. A potentially fatal complication of hemorrhagic colitis that occurs in 2–7% of infected patients (usually the young or very old) is hemolytic uremic syndrome. Because the toxin can kill the endothelial cells lining the tiny blood vessels of the glomerulus of the kidney, severe kidney damage can result such that the patient may permanently require dialysis after recovery. About 5% of hemolytic uremic syndrome cases are fatal.

A particularly nasty subgroup of extracellular gram-negative bacteria are the spirochetes, including members of the genera *Borrelia*, *Leptospira*, and *Treponema*. *B. burgdorferi* is the pathogen that causes Lyme disease. Lyme disease, named after the Connecticut town in which it was discovered, is characterized by a skin rash often accompanied by acute arthritis and/or carditis. The pathogen is transmitted to humans by way of tick bites. Various species of *Leptospira* cause leptospirosis, a disease characterized by acute fever that can lead to liver and kidney damage. Contact with urine from leptospiruric animals (including pet dogs) is the primary route of transmission to humans. Syphilis is a sexually transmitted disease caused by invasion of the genital mucosae by *Treponema pallidum*. The pathogen replicates rapidly at first but then can enter a latent phase. Recurrences erupting several years after the initial infection can lead to damage of the aorta, central nervous system, eyes, and ears.

An organism that has become a topic of much public concern lately is the organism that causes anthrax, *Bacillus anthracis*. This large, gram-positive extracellular bacterium is a common pathogen of hoofed livestock and range animals. It lives in the soil of farm and range lands and produces spores

that can survive in soil for decades. While *B. anthracis* infection occurs commonly in livestock, it rarely infects humans. Indeed, prior to the bioterrorism attacks through the postal system in the United States in the fall of 2001, the last case of inhalation anthrax in the United States was recorded in 1976. When *B. anthracis* does infect a human, the resulting anthrax disease takes one of three forms: inhalation (the most lethal), cutaneous, and gastrointestinal. If left untreated, any type of anthrax can lead to septicemia and death. Cutaneous anthrax, the most common form, usually occurs after introduction into a skin abrasion of bacteria from contaminated meat, wool, hides, or leather of infected animals. Skin lesions develop within 12 days of exposure and become necrotic. Patients may also experience fever and lymph node swelling. Gastrointestinal anthrax occurs after consumption of raw or undercooked contaminated meat. Severe abdominal symptoms, including bloody diarrhea, vomiting, and fever, occur within a week of exposure. Inhalation anthrax is contracted by breathing aerosolized spores of *B. anthracis*. It was originally thought that thousands of spores had to be inhaled to cause disease, but that number has been revised downward in light of the deaths of mail workers exposed to more modest bacterial loads. Within 7 days of exposure to aerosolized *B. anthracis*, symptoms resembling a viral respiratory illness ensue that rapidly progress to shock, respiratory failure, and sometimes meningitis. Death occurs in 75% of cases, even with aggressive antibiotic therapy.

### ii) Host Damage Caused by Extracellular Bacteria

Many of the disease symptoms caused by extracellular bacteria can be attributed to their production of toxins. *Exotoxins* are toxic proteins actively secreted by both gram-positive and gram-negative bacteria, while *endotoxins* are the lipid portion of lipopolysaccharides embedded in the walls of gram-negative bacteria. A given bacterial species may supply both exotoxins and endotoxins. For example, many strains of *E. coli* produce one or more exotoxins and contain the LPS endotoxin in their cell walls. *Pseudomonas aeruginosa* also contains LPS but secretes a different cytolytic exotoxin called exotoxin A.

**iia) Direct toxicity caused by exotoxins.** Different exotoxins cause disease by different means and in different locations, depending on the proclivities of the individual bacterial species. For example, infection with *V. cholerae* results in the local release of an exotoxin that binds to gut epithelial cells. A massive release of electrolytes and tissue fluids is induced that is manifested as the severe diarrhea that characterizes cholera. Although they are derived from extracellular bacteria, many bacterial exotoxins have the ability to translocate into mammalian cells and wreak havoc on intracellular processes. The diphtheria exotoxin secreted by *Corynebacterium diphtheriae* travels the body systemically and is absorbed by cells of the heart and peripheral nervous system. The toxin inhibits protein synthesis in these cells, leading to myocarditis and neuritis. The diphtheria exotoxin also promotes colonization of the throat by the bacterium, which provokes an acute inflammatory response resulting in severe respiratory obstruction. As mentioned previously, *C. botulinum* produces a neurotoxin that blocks the transmission of nerve impulses to the muscles, resulting in paralysis. This toxin is much feared as a potential biological weapon because a dose of less than 1 μg is fatal to humans. Another *Clostridium* species, *Clostridium tetani*, synthesizes a neurotoxin that causes uncontrollable muscle contractions. Other exotoxins trigger specific host cell necrosis, such as the leukocidin produced by *S. aureus* that is toxic to granulocytes. Another example is the exotoxin produced by *E. coli* O157:H7, which causes severe hemorrhaging because it blocks protein synthesis within vascular endothelial cells and kills them. *B. anthracis* produces two exotoxins called *lethal toxin* and *edema toxin* that damage phagocytes in an unknown way. Lethal toxin is composed of a zinc protease called *lethal factor* and a protein called *protective antigen*, while edema toxin is composed of an adenylate cyclase called *edema factor* plus protective antigen.

**iib) Immunopathic damage.** Extracellular bacteria furnish many examples of situations in which the adaptive immune response to the infection results in immunopathic disease. In some cases, the destruction is caused by endotoxins, and in others an exotoxin is responsible. It is also becoming apparent that prolonged infection with many organisms can sometimes lead to the production of antibodies or activation of T cells that cross-react with self tissues, causing inflammation and possibly autoimmune disease. The concept of "molecular mimicry," in which a pathogen antigen resembles a self antigen enough to provoke an attack on self tissues by anti-pathogen antibodies and T cells, is explored fully in Chapter 29.

Damage to a host caused by an endotoxin is always immunopathic and occurs by the same mechanism in each case. As described in Box 22-1, the cell wall of a gram-negative bacterium contains lipopolysaccharides composed of the endotoxin Lipid A and one of several *O-linked polysaccharides* (O-PS). Not only are the O-PS moieties often specific to a particular bacterial species but they are also immunogenic, leading to their designation as "O-specific antigens." When the immune system responds to these antigens and destroys a gram-negative bacterium, its membrane disintegrates and Lipid A is released into the blood or gastrointestinal tract. The presence of Lipid A induces a massive release of cytokines (particularly TNF) that cause high fever and endotoxic shock (refer to Box 17-2). For example, in the case of *Helicobacter pylori* infection, Th1 cells responding to this organism produce large amounts of pro-inflammatory cytokines (principally TNF and IFNγ) that exacerbate the injury to the gastric lining and promote the formation of stomach ulcers. Thus, current treatments for ulcers include a course of antibiotics, to tackle the problem at its root.

An effect similar to endotoxic shock can result from the polyclonal activation of $CD4^+$ T cells by bacterial exotoxins that are superantigens (refer to Box 14-2). The reader will recall that T cell activation by a superantigen does not depend on either specific peptide or costimulation. The simultaneous activation of a wide variety of different T cell clones results in the production of huge quantities of pro-inflammatory cytokines. In addition, some of the activated T cell clones may be directed against self antigens. These clones would normally be held anergic in the presence of the self antigens in the host, but may go on to attack self tissues after non-specific activation by the bacterial exotoxin.

## II. EFFECTOR MECHANISMS

The major mechanisms by which the immune system eliminates extracellular bacteria are summarized in Figure 22-2.

### i) Humoral Defense

Extracellular bacteria by definition cannot routinely "hide" within host cells. Thus, the antibodies of the humoral response are generally highly effective against these species. Indeed, patients who are deficient in immunoglobulins (*agammaglobulinemic*) and infected with an extracellular bacterium can be successfully treated by a passive transfer of specific anti-bacterial antibodies. For a normal, healthy individual, the polysaccharides present in bacterial cell walls make perfect Ti antigens for B cell activation, while other bacterial components supply Td antigens that induce primarily a Th2 response and help for B cells recognizing bacterial Td antigens. Anti-bacterial antibodies of the polymeric IgM isotype dominate in the vascular system, while IgG antibodies, because of their smaller size, are able to access the tissues.

As we learned in the first section of this book, antibodies can neutralize bacteria by physically blocking them from attaching to host cell surfaces. Even though they do not need to enter host cells for replication, most extracellular bacteria adhere to host cells to avoid being swept off or out of the host by skin sloughing or movement of the intestinal contents. Small hair-like molecules called *pili* on the exterior surface of the bacterium allow it to stick to glycoproteins on host cells via a lectin-like mechanism. Different bacterial species bear different types of pili that interact with different ligands on host cells in different locations. Thus, the nature of the pili can determine where in the body the bacteria will attempt to establish colonization. Binding of antibody to a protein in the pili can inhibit bacterial adherence to a host cell, allowing the invader to be flushed away.

Antibodies can also serve as opsonins, coating the surface of the bacterium such that it becomes a more attractive target for phagocytosis mediated by neutrophils and macrophages that express Fc receptors. Extracellular bacteria are generally very vulnerable to the killing mechanisms that operate within a phagosome. Changes in pH within the phagosome, defensins, and the action of the reactive oxygen intermediates (ROIs) and reactive nitrogen intermediates (RNIs) all contribute to bacterial killing.

Antibodies are also made against the exotoxins used by bacteria to kill or disable host cells. *Antitoxins* are antibodies that bind to the toxin itself (not the bacterium producing it) and either cause its rapid removal or block its active site. Effective antitoxins are often of the IgG isotype because of the ability of this class to diffuse into the tissues. Antitoxins can also act as opsonins and enhance the clearance of the toxin by phagocytes. Sometimes secretory IgA can act as an antitoxin by preventing a toxin from making contact with and damaging a body tract such as the airway. If the toxin is the sole element causing disease in the host, the production of the antitoxin alone will be enough to restore health. For example, human resistance to tetanus relies on the presence of antibodies directed against the exotoxins produced by *Clostridium tetani*. The tetanus *toxoid* vaccination that we receive as infants (followed by tetanus boosters every 10 years) is a preparation of inactivated tetanus exotoxin that serves to induce and maintain the production of neutralizing antitoxin antibodies (see Ch.23). Not surprisingly, antigenic variation can influence antitoxin effectiveness. For example, the composition of a bacterial exotoxin may vary slightly among strains such that an antitoxin generated in an exposure to one strain may not recognize the epitopes of exotoxin produced by a later infection with a second strain. The advantage of secondary response levels of antibody is lost, as the host is forced to mount a primary response to the new epitope.

### ii) Complement

All three pathways of complement activation can be brought to bear on extracellular bacteria. The binding of antibody to a pathogen allows MAC-mediated destruction of some bacterial species by the classical pathway of complement activation. The alternative pathway can be activated in the absence of antibody by peptidoglycan in gram-positive cell walls or LPS in gram-negative cell walls. Because of their more delicate membrane structure, gram-negative bacteria usually succumb more rapidly to complement-mediated lysis than gram-positive bacteria. Lastly, the lectin pathway is activated by the binding of MBL to the distinctive sugars arranged on the surfaces of bacterial cells. As we saw in Chapter 19, complement-mediated lysis is particularly crucial for defense against the *Neisseria* group of gram-negative extracellular bacteria. Patients lacking the terminal complement components are still able to resist most bacterial infections, but not those initiated by these pathogens.

### iii) Th Cells

Resolution of an infection by an extracellular bacterium cannot always be directly equated with elimination by the antibodies of a Th2 response. For example, infection with the extracellular bacterium *Yersinia enterocolitica* is associated primarily with a Th1 response, an interesting observation since this pathogen was originally thought to replicate intracellularly. Other particularly invasive or insidious extracellular pathogens, such as the spirochetes, provoke a combination of Th1 and Th2 responses. For example, immune responses to *B. burgdorferi* and *T. pallidum* have both humoral and inflammatory cell-mediated components. Even so, these organisms are difficult to eradicate, and development of a secondary response adequate to protect against re-infection develops only slowly, if at all. A clue to this intransigence may arise from the study of immune responses against *Borrelia recurrentis*, which causes recurring febrile episodes. Most of these bacteria are eliminated by the humoral response, but a few mutate just at the very end of the acute infection and escape immune system elimination.

### iv) Mast Cells

Mast cells play a prominent role in defense against extracellular bacteria, in particular against gram-negative species expressing a bacterial adhesin protein called FimH. Mast cells lurking in the MALT both phagocytose the offending bacteria and release inflammatory mediators such as TNF, leukotrienes (especially LTβ4), IL-6, and histamine. One of

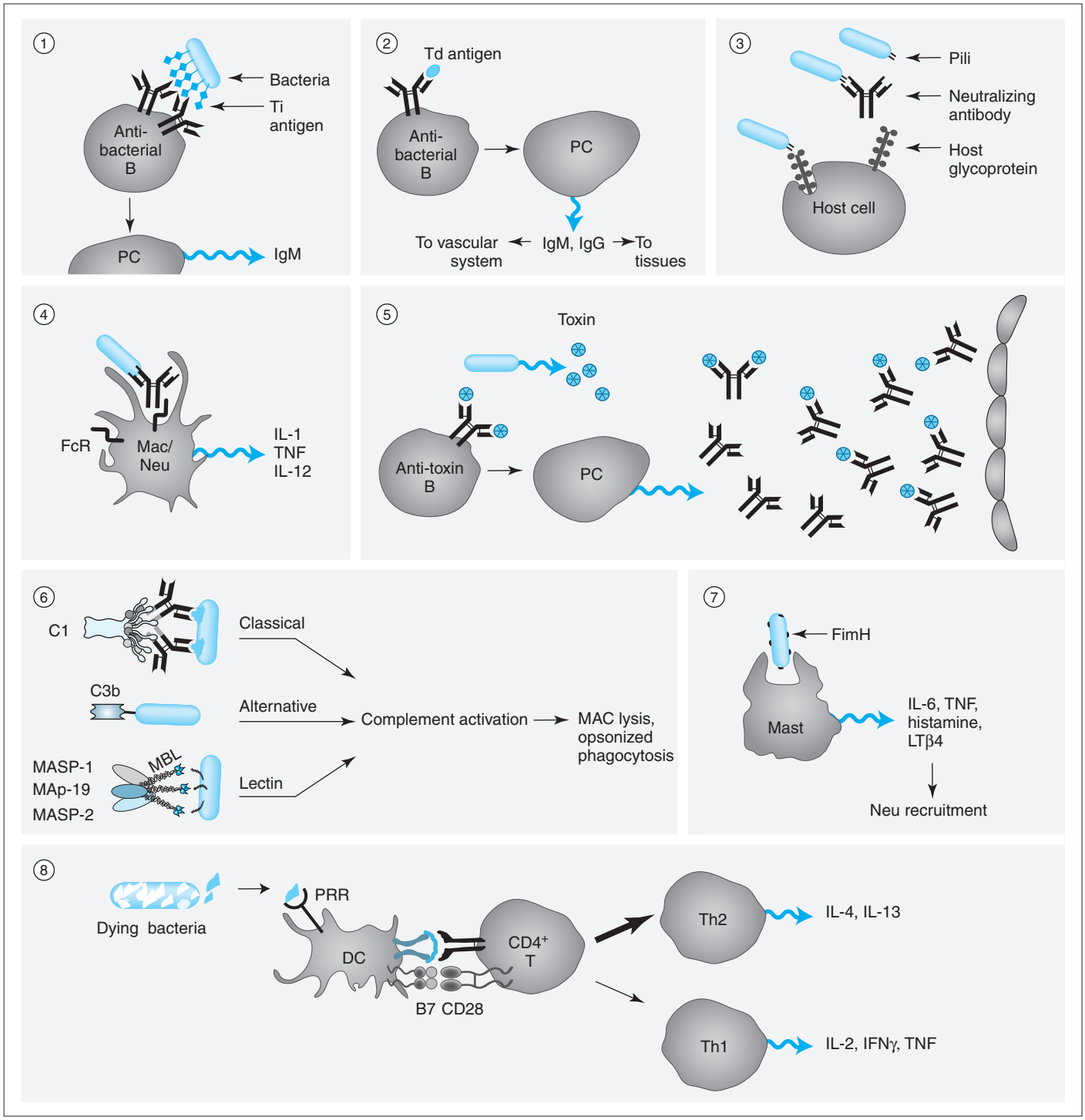



Figure 22-2

**Major Mechanisms of Immune Defense against Extracellular Bacteria**
(1) Polysaccharides on bacteria can act as Ti antigens and activate B cells that produce anti-bacterial IgM antibodies. (2) Other bacterial components can act as Td antigens and activate additional B cells that produce anti-bacterial IgM and IgG antibodies. (3) Neutralizing antibodies recognizing bacterial pili block access of these bacteria to host cell glycoproteins. (4) Neutralizing antibodies bound to bacteria or their components are recognized by FcR on macrophages and neutrophils. Once activated, these cells initiate receptor-mediated endocytosis of the pathogen and pro-inflammatory cytokine production. (5) A B cell recognizing a bacterial toxin produces neutralizing anti-toxin antibodies that prevent the toxin molecules from damaging the cell surfaces. (6) Bacteria that have been bound by antibody plus C1, C3b, or MBL activate complement via all three pathways. (7) Mast cells activated by an encounter with a FimH-expressing bacterium release histamine and pro-inflammatory cytokines that lead to the recruitment of neutrophils. (8) Bacterial components captured by DCs are processed and presented to $CD4^+$ T cells. Th2 differentiation, which supports antibody production, is generally favored over Th1 differentiation.

the primary functions of these mediators is to promote the release of IL-8 and thus recruit neutrophils to the site of pathogen invasion. LTβ4 is itself a powerful chemoattractant for neutrophils, and TNF enhances the microbicidal activities of these cells. The importance of mast cells for defense against gram-negative bacteria has been convincingly demonstrated in W/W mice, mutant animals that lack mast cells. Whereas wild-type mice were able to shrug off a peritoneal challenge with a FimH-expressing enterobacterium, over 80% of W/W mice died after infection with this pathogen. If the W/W mice were injected with TNF, neutrophil emigration into the site of infection increased and the mice survived.

## III. EVASION STRATEGIES

Extracellular bacteria have evolved mechanisms, some general and some specific, to avoid elimination by antibodies, phagocytosis, and/or complement (Table 22-5).

### i) Avoiding Antibodies

Many species of gonococci enhance their chances of maintaining adhesion to a non-phagocytic host cell by routinely and spontaneously altering the expression of their pili. A large number of normally silent loci in the gonococcal genome allow frequent gene conversion (refer to Box 21-3) between the active pilin locus and the silent loci, resulting in an altered nucleotide sequence that translates into an adhesion protein of modified amino acid sequence. Neutralizing antibodies directed against epitopes in one version of the pilin protein may not "see" the new pili, allowing the bacterium to maintain its adhesion and establish an infection.

Certain pathogenic bacteria secrete proteases that cleave antibody proteins. For example, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, and *Streptococcus sanguis* express IgA-specific proteases that degrade both sIgA antibodies in the blood and secretory IgA antibodies protecting the mucosae. These proteases are preferentially directed against IgA1 and usually cleave this molecule in its hinge region. In this case, the antibody loses the domains required for binding to Fc receptors, limiting its effector functions. In addition, the truncated antibody may bind to the pathogen using its Fab region but lacks the ability to agglutinate the microbes. A similar protease from *Proteus mirabilis*, which causes urinary tract infections, can cleave IgA1, IgA2, and IgG.

Table 22-5 **Evasion of the Immune System by Extracellular Bacteria**

| Immune System Element Thwarted | Bacterial Mechanism |
|---|---|
| Antibodies | Alter expression of surface molecules<br>Secrete anti-Ig proteases |
| Phagocytosis | Block binding of PRRs and complement receptors to bacterial capsule<br>Inject bacterial protein that promotes uptake by non-phagocytes<br>Inject bacterial protein that disrupts phagocyte fusion and function |
| Complement | Lack a protein C3b can bind to<br>Express long-chain O-antigen in lipopolysaccharide<br>Degrade C3b<br>Block alternative C3 convertase formation<br>Block terminal component addition<br>Shed activating surface moieties, immune complexes, or partially assembled MACs<br>Capture host RCA proteins<br>Alter expression of host RCA proteins and complement receptors<br>Express molecules that mimic complement components or RCAs<br>Inactivate anaphylatoxins<br>Induce host production of poor complement-fixing Abs or Abs that block C3b binding |
| APCs | Block maturation or migration of DCs |

### ii) Avoiding Phagocytosis

Many extracellular bacteria, including *N. meningitidis*, *S. pneumoniae*, and *H. influenzae*, avoid phagocytosis by covering themselves in a polysaccharide coating called a *capsule* ("encapsulated bacteria"). The capsule confers a charge and hydrophilic character to the bacterial surface that inhibits binding to phagocyte receptors. C3b may still attach to the bacterial surface, but the capsule sterically interferes with the binding of the phagocyte's receptors to the C3b. Instead, the host must synthesize opsonizing antibodies to allow a phagocyte to successfully attach to these invaders. Encapsulated bacteria therefore have more time (several days) than unencapsulated bacteria to reproduce undisturbed, making them more dangerous pathogens. For example, alveolar macrophages cannot engulf the encapsulated bacterium *S. pneumoniae* unless it is opsonized, allowing early establishment of the bacteria in the lungs. Eventually, antibodies to components of the capsule are made that facilitate destruction of the bacteria by complement-mediated lysis. However, if this tactic is not rapid enough, pneumonia can result. Those with deficits in the humoral response, such as the very young, the very old, and the immunocompromised, are especially susceptible to infections with encapsulated bacteria. To protect healthy adults at particular risk of exposure to these pathogens, a vaccine has been developed that contains the capsule components of several species (see Ch.23). A dangerous wrinkle in the capsule story is that some streptococci have capsules made of substances that are chemically identical to human components, such as hyaluronic acid. Antibodies raised against such substances might have the unwelcome collateral effect of promoting autoimmunity. Such considerations have mandated caution in vaccination against these pathogens.

Some extracellular bacteria avoid capture by hostile phagocytes by entering non-phagocytes such as epithelial cells and fibroblasts. The pathogens inject bacterial proteins into the host cell that promote cytoskeletal rearrangements facilitating macropinocytosis. The bacteria are taken up by the non-phagocytes and thus gain unmolested access to the tissues. Receptor-mediated endocytosis can be exploited in a similar

way. For example, enteropathogenic *E. coli* injects the Tir (*translocation intimin receptor*) protein into the cytoplasm of epithelial cells. The act of injection induces a rearrangement of the host cell cytoskeleton that results in Tir being fixed in the plasma membrane, ready to act as a receptor for the surface protein intimin on the bacterium. Receptor-mediated endocytosis ensues, importing the bacterium into the non-phagocytic cell.

Some species of *Yersinia* have direct anti-phagocyte activity. *Y. enterocolitica* is an extracellularly replicating enteric pathogen that targets the FAE of the PPs and causes severe diarrhea. This bacterium forestalls phagocytosis by attaching to the exterior of a looming macrophage and injecting a set of so-called *Yop proteins* into the macrophage cytoplasm. Among the Yop proteins is YopH, a phosphatase that binds to certain tyrosine-phosphorylated proteins in the macrophage that are thought to be involved in intracellular signaling and the operation of its actin cytoskeleton. When YopH dephosphorylates these host proteins, disruptions in intracellular signaling block integrin-mediated phagocytosis of the bacterium. A different Yop protein appears to induce the apoptosis of macrophages, at least *in vitro*.

### iii) Avoiding Complement

Evasion of the complement system is a time-honored strategy employed by a vast array of pathogens. Specific methods include avoiding recognition by complement proteins, removing any attached complement proteins, exploiting various host RCA (regulators of complement activation) proteins to either inactivate the complement cascade or gain access to a host cell, and expressing molecules that resemble host complement components or RCAs. Many anti-complement strategies employed by extracellular bacteria are shared by intracellular bacteria, viruses, and parasites. Box 22-3 explores in depth several of

**Box 22-3. Avoidance of the complement system by pathogens**

The complement system is a vital contributor to the defense of the host in both the innate and adaptive immune responses. Naturally, therefore, pathogens have devoted much time and energy to devising ways to confound the complement system, or to make use of it for their own ends. Pathogens can avert complement-mediated destruction by presenting a cell surface that does not permit deposition of C3b (avoiding recognition); by shedding incipient complexes as they form; by destroying or inhibiting various complement proteins; by acquiring host RCA proteins; and/or by expressing molecules that closely resemble host RCA proteins. At any one time, a given pathogen may be able to use just one or several of these mechanisms to protect itself.

A pathogen can avoid complement recognition if the surface moieties it expresses do not bind C3b or antibody well or at all. The surface glycoproteins of some parasites and capsules of some bacteria fit this description. For example, some species of gram-negative bacteria avoid detection by expressing LPS residues with unusual side chain sugars. These sugars mask the LPS determinants that would normally bind to C3b and activate complement via the alternative pathway. Another avoidance mechanism is the shedding of activatory surface moieties or immune complexes, as occurs for the parasites *Schistosoma mansoni* and *Trypanosoma cruzi*, respectively. Some organisms express molecules that disrupt one or more complement activation pathways. For example, Protein H expressed by *Streptococcus pyogenes* binds to C1 but does not allow the cascade to proceed, competitively inhibiting the classical pathway. Several members of the poxvirus and herpesvirus families secrete proteins that block formation of the alternate C3 convertase. Even if either C3 or C1 has succeeded in initiating complement activation, many pathogens, such as the bacterium *Escherichia coli* and the parasite *Leishmania major*, respond by shedding the complex, particularly at the MAC formation stage. Proteolytic degradation and phosphorylation of complement components are also used by many organisms to derail MAC assembly before it can go too far. Some organisms (like certain *Borrelia* and *Salmonella* species) express proteins that inhibit the terminal complement proteins, so that C6 deposition is blocked or C9 polymerization is inhibited.

Another effective strategy for blocking complement-mediated destruction is to acquire the host RCA proteins that normally protect host cells. Budding viruses take sections of the host cell membrane with them as they emerge, and embedded in the membrane are host RCA proteins. DAF and MIRL are acquired by HIV and vaccinia in this way. Other organisms express molecules that adsorb soluble RCA proteins from the host plasma. The host RCA proteins retain their regulatory activity even when bound to a bacterial surface, so that the alternative pathway of complement activation is inhibited. For example, the fluid phase RCA proteins Factor H and C4bp can be bound to the M proteins of *Streptococcus pyogenes*. The C4bp–M protein binding is completely sufficient to halt complement-mediated opsonization and phagocytosis. C4bp has also been shown to bind to all known strains of *Bordetella pertussis*. In *Neisseria gonorrhea*, both surface sialic acids and an outer membrane protein called *porin* can bind Factor H.

Altering the expression of host RCA and CR proteins is another means of countering complement. Some intracellular pathogens induce new expression of RCA proteins to block complement-mediated destruction, thus preserving the pathogen's chosen home. For example, DAF and MCP expression are upregulated on cells infected with human CMV, and a modified form of DAF appears on cells infected with *Plasmodium berghei*. HIV downregulates the expression of CR1 and CR2 on the infected host cell, reducing opsonized phagocytosis. HIV also reduces the expression of C5aR by monocytes, decreasing the chemotaxis of these cells to sites of infection and inflammation. A related mode of pathogen defense is the expression of molecules that resemble host RCA proteins. Proteins with similar structures and functions to mammalian CR1, DAF, MCP, C4bp, and MIRL have been described in viruses such as vaccinia, KSHV, and HSV, as well as in parasites such as *Schistosoma*, *Entamoeba*, and *Trypanosoma*. Studies of *Herpesvirus saimiri* have shown that this virus has apparently acquired the cDNA for mammalian MIRL and incorporated it into the viral genome. The virus induces the expression of a GPI-anchored protein in the host cell membrane that closely resembles monkey MIRL and protects against MAC-mediated lysis.

Lastly, some bacteria and parasites proteolytically inactivate complement cascade products such as the anaphylatoxins C5a and C3a. A lack of these molecules dampens the inflammatory response and gives the pathogen more time to multiply or hide. For example, a membrane-bound enzyme that removes the C-terminus of C5a is expressed on the surface of streptococci.

these tactics from the point of view of a particular complement system component.

Perhaps the simplest approach to evading complement has been taken by the organism causing syphilis, *T. pallidum*. The outer membrane of this gram-negative bacterium is strikingly devoid of transmembrane proteins, offering almost no place suitable for the deposition of complement components. Other bacteria avoid complement-mediated lysis due to the nature of the O-PS of their cell wall lipopolysaccharides. Classical complement activation mediated by antibodies directed against the O-PS can eliminate a bacterium. However, if the polysaccharide chains of the O-PS are very long and project a great distance from the bacterial cell wall, the MAC cannot assemble on the bacterial surface and the invader is spared. Bacteria expressing O-PS with short polysaccharide chains are vulnerable to complement-mediated lysis.

Many extracellular bacteria secrete enzymes that inactivate various steps of the complement cascade. For example, group B streptococci, which cause neonatal meningitis, contain sialic acid in their cell walls, which can block alternative complement activation by degrading C3b that is attempting to attach to the pathogen surface. Several streptococci species also produce M proteins that can bind to the fluid phase RCA protein Factor H and fix it onto the bacterial surface. In its hijacked site, the recruited Factor H makes any C3b that has attached susceptible to degradation, and thus protects the microbial surface. Even if a host develops antibodies to a particular M protein, these antibodies tend not to be cross-reacting, and there are more than 80 different types of M proteins. This means that the host will likely still be vulnerable to a subsequent infection with a different streptococcal strain expressing a different M protein.

Gonococci and meningococci may have the most complex approach to avoiding death by complement. These organisms induce the host to produce "blocking antibodies" that recognize particular proteins on the surface of the bacteria but are of isotypes that are poor at fixing complement, such as IgA. The blocking antibodies compete with complement-fixing antibodies for binding to the bacterial surface, and prevent bacterial destruction by complement-mediated lysis. In addition, steric hindrance by the blocking antibodies interferes with the deposition of C3b and thus thwarts the alternative pathway of complement activation.

## C. Immunity to Intracellular Bacteria

### I. WHAT ARE INTRACELLULAR BACTERIA?

Intracellular bacteria have evolved the ultimate escape from phagocytes, complement, and antibodies: they move right inside the host cell and complete their reproduction out of reach of these host defenses. Some species infect non-immune system host cells such as hepatocytes and epithelial and endothelial cells, while others show a strong predilection for macrophages. While some intracellular bacterial species cannot survive outside of a host cell, others merely make intracellular replication a preference. Like extracellular bacteria, most intracellular bacteria access the host via breaches in the mucosae and skin, but some are introduced directly into the bloodstream by the bites of vectors such as ticks, mosquitoes, and mites.

Intracellular bacteria generally enter the host cell by receptor-mediated endocytosis and are thus first confined to intracellular vacuoles. Some species remain in the vacuolar compartment, while others leave it to take up residence in the cytosol. Because of their desire to replicate within a host cell and keep it alive for this purpose, intracellular bacteria are generally not very toxic to the host and do not produce tissue-damaging bacterial toxins. Because of their low toxicity, infections with intracellular bacteria have extended incubation times. However, their intracellular lifestyle makes infections with these organisms difficult to resolve completely. These factors mean that infections with intracellular bacteria tend to result in chronic or recurrent rather than acute disease. Cell-mediated immunity is crucial for combating infections by intracellular bacteria. Thus, immunocompromised individuals who lack the ability to mount cell-mediated immune responses are particularly at risk for infection with these pathogens.

Important species of intracellular bacteria belong to the *Salmonella*, *Listeria*, *Brucella*, *Rickettsia*, and *Legionella* genera (Table 22-6). *Salmonella typhi* is an encapsulated, gram-negative enterobacterium that causes typhoid fever in humans. *Salmonella typhimurium* causes the equivalent of typhoid fever in mice. Humans can be infected by *S. typhimurium* if they eat improperly cooked meat from infected cattle. In this case, the infection results in food poisoning. Like all *Salmonella* species, *S. typhi* and *S. typhimurium* transcytose across M cells in the mucosae. However, unlike the extracellular *Salmonella enteritidis* bacteria, which cause poultry-related food poisoning, *S. typhi* and S. *typhimurium* invade macrophages to replicate and thus are intracellular pathogens. Another food-borne intracellular pathogen is *Listeria monocytogenes*, a gram-positive rod that infects the cytoplasm of macrophages and hepatocytes. *L. monocytogenes* causes meningeal or systemic

Table 22-6 **Examples of Intracellular Bacteria and the Diseases They Cause**

| Organism | Disease |
|---|---|
| *Brucella* (various) | High fevers, brucellosis |
| *Legionella pneumophila* | Legionnaire's disease |
| *Listeria monocytogenes* | Listeriosis |
| *Mycobacterium leprae* | Leprosy |
| *Mycobacterium tuberculosis* | Tuberculosis |
| *Rickettsia rickettsii* | Rocky Mountain spotted fever |
| *Salmonella typhi* | Typhoid fever |
| *Salmonella typhimurium* | Typhoid fever-like disease in mice, food poisoning in humans |
| *Shigella flexneri* | Enteric disease |

infections most often in sheep and cattle but sometimes in humans. Unlike most bacteria, *Listeria* can multiply quite successfully at low temperatures, meaning that the infection continues to grow in contaminated food even when it is refrigerated. *Listeria* infection has also been associated with miscarriages and stillbirths in humans and cattle. Mouse models of *Listeria* infection (listeriosis) have been very helpful in discovering how the immune system combats intracellular pathogens (see later). However, for all its use in experimental models, it is not yet clear how *L. monocytogenes* makes its way across the intestinal barrier into the liver and spleen and finally across the blood–brain barrier. Various *Brucella* species are transmitted to humans via contact with animals and result in high fevers. *Rickettsia rickettsii* is transmitted into the bloodstream via tick bites and proceeds to attack endothelial and smooth muscle cells, causing Rocky Mountain spotted fever. *Legionella pneumophila*, the relatively recently identified pathogen that causes the lung disorder known as "Legionnaires' disease," replicates only within macrophages.

More familiar examples of intracellular bacterial pathogens belong to the *Mycobacterium* genus. *M. tuberculosis* causes tuberculosis, and *M. leprae* infection leads to leprosy. The mycobacteria do not produce bacterial toxins and cause only mild inflammation on their own. However, these bacteria are extraordinarily hard to remove, and it is the prolonged host response to a mycobacterial infection that causes most of the tissue damage. Consider *M. tuberculosis*, a slow-replicating (12 hour) aerobic bacillus that gravitates to well-aerated tissues such as the lung. Tubercular disease associated with *M. tuberculosis* is caused not only by inflammatory damage in the lungs induced by the bacteria but also by the host's adaptive immune response. The persistence of the bacteria summons activated T cells that supply products and contacts that hyperactivate macrophages. The hyperactivated macrophages in turn secrete copious amounts of pro-inflammatory cytokines that result in lung damage. In addition, the macrophages may eventually form granulomas to wall off the bacteria. These granulomas are the "tubercles" (lumps or knobs) on the lungs that give the disease its name. Eventually, the inner circle of macrophages in the tubercles breaks down and the bacilli are released. If the body's defenses can arrest the disease effectively at this point, the lesion calcifies and becomes visible in X-rays as scarring on the lung. If the disease is not arrested, the bacilli persist and may become dormant for months or even years. At some point, when the immune system is weakened due to poor health, malnutrition, or other infections, the bacilli in the center of the tubercle may be able to proliferate again. However, in this situation, bacterial multiplication takes place outside macrophages for the first time. Should the tubercles rupture, millions of bacteria are released into the lungs. The patient becomes highly contagious at this point due to the release of bacilli during coughing episodes. From the lungs, the bacteria disseminate throughout the patient's body and induce the formation of seed-sized tubercles in infected tissues. Body defenses may be overwhelmed, leading to the weight loss, coughing (often with blood), and loss of vigor known collectively as "consumption."

Treatment of TB is a challenge, because the slow growth habit of the bacterium and its ability to hide in macrophages or other locations for extended periods mean that the patient is often asymptomatic. Indeed, 90% of individuals infected with *M. tuberculosis* remain clinically healthy. An asymptomatic patient is sometimes reluctant to continue with the long-term course of antibiotics needed to successfully treat the disease. A classical "TB test," involving the cutaneous injection of tuberculin antigen (often called *purified protein derivative*; PPD) into the skin, indicates whether an individual is or has been infected with *M. tuberculosis* (and thus may still be harboring the bacterium) (Plate 22-1). A pronounced,

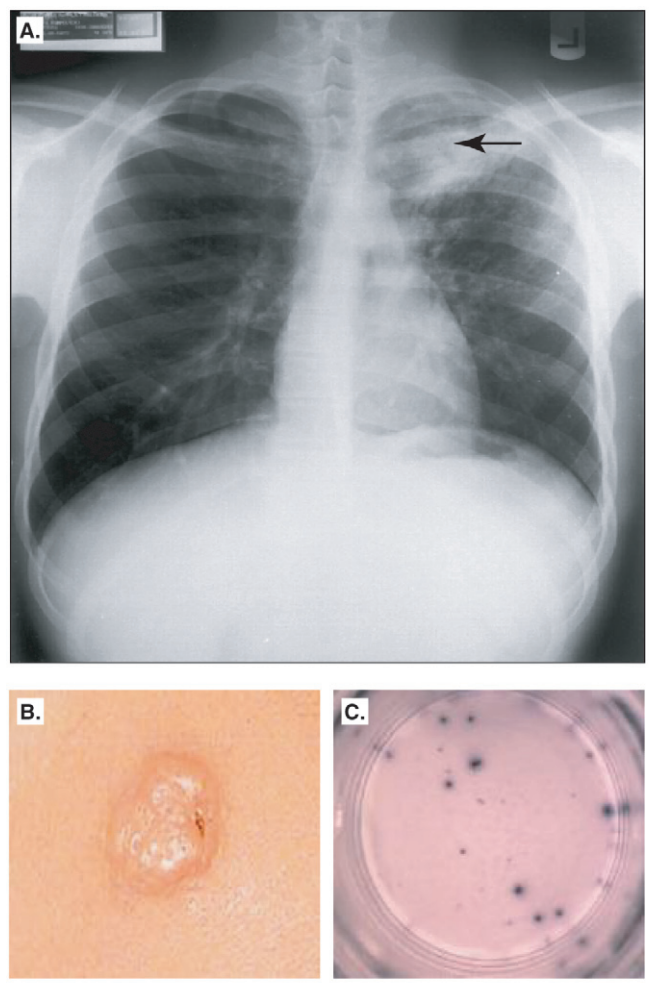



Plate 22-1

**Tuberculosis**

(A) Chest X-ray of an adolescent with tuberculosis showing disease of the upper lobe of the lung and early formation of a cavity (arrow). Courtesy of Dr. Ian Kitai, The Hospital for Sick Children, Toronto. (B) Tuberculin skin test based on a delayed type hypersensitivity in response to a crude mixture of *M. tuberculosis* proteins. (C) *Ex vivo* assay detecting secretion of IFN$\gamma$ by individual peripheral blood T cells after stimulation with specific mycobacterial antigens. Courtesy of Dr. Ajit Lalvani, Nuffield Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford. [Lalvani A. *et al*. (2001) Rapid detection of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. *American Journal of Respiratory and Critical Care Medicine* 163, 824–828.]

reddened reaction on the skin around the inoculation site after about 48 hours is a DTH response: memory T cells responding to the inoculated antigen activate macrophages that produce cytokines inciting local inflammation. In the very young, a positive TB test probably means that an active TB infection is occurring. In older people, a positive test can result from either an active infection, or from a previous infection that has been cleared, or from vaccination. In these cases, follow-up testing is done by X-ray examination of the lungs, which can distinguish between an active and a calcified lesion. There may also be an attempt to culture the bacterium from the patient. A new generation of TB tests under development includes variations of ELISA assays that measure the production of IgM antibodies or activation of T cells directed against epitopes specific to *M. tuberculosis*.

Healthy individuals living in generally sanitary environments (such as those in most of the developed world) are usually able to successfully fight off *M. tuberculosis*. However, the immune systems of those living in developing countries and socio-economically depressed areas of developed countries may be stressed just enough to put them at increased risk for TB. Attempts have been made to slow the spread of TB in such populations by antibiotic treatment and vaccination programs (see Ch.23). However, it takes at least 6 months of intense chemotherapy to cure a TB patient because of the limited effectiveness of available drugs against entrenched *M. tuberculosis*. Moreover, poor patient compliance with this stringent regime has led to the rise of multi-drug resistant strains of *M. tuberculosis*. In addition, administration of the *bacillus Calmette-Guérin* (BCG) strain of *Mycobacterium bovis* used as a vaccine against TB does not always induce complete cell-mediated immunity. These factors have led to a maddening persistence and recurrence of TB in susceptible populations. It is estimated that there are 2 billion people worldwide who are latently infected with *M. tuberculosis*, and that about 5–10% of these experience re-activation of the bacterium, leading to active disease. Tuberculosis kills about 1.6 million people per year worldwide.

*M. leprae*, the organism responsible for leprosy, grows even more slowly than *M. tuberculosis*, taking about 12 days to replicate in humans. Because the optimal growth temperature for this organism is 30 °C, *M. leprae* preferentially establishes infections just under the skin. The bacterium infects the peripheral nerves in this location and causes visible skin lesions. Two clinical types of leprosy have been identified based on disease phenotype and the strength of the cell-mediated immune response (Plate 22-2). "Tuberculoid leprosy," also called "neural leprosy," is associated with a vigorous cell-mediated immune response. The lesions, which are confined to the skin, contain moderate numbers of bacteria. However, the damage to peripheral nerves causes loss of nerve function that frequently leads to injury. Anti-mycobacterial antibody titers are low in these patients. However, if the infected patient mounts only a weak cell-mediated response to the organism, tuberculoid leprosy may become "lepromatous leprosy." This form of the disease is characterized by severe skin lesions containing large numbers of bacteria that can destroy underlying bone and cartilage, leading to loss of structures such as the fingers and the nose. Anti-mycobacterial antibody titers are high in these patients but are ineffective in controlling the disease.

## II. EFFECTOR MECHANISMS

The major mechanisms by which the immune system eliminates intracellular bacteria are summarized in Figure 22-3.

### i) Neutrophils

Early infections by intracellular bacteria are controlled by killing mechanisms associated with neutrophil phagocytosis. For example, infection of epithelial cells by *L. monocytogenes* generally induces the apoptosis of these cells, releasing chemoattractant molecules that draw neutrophils to the area. Bacteria released from the dying cells are phagocytosed by the neutrophils, whose defensins and powerful respiratory burst usually kill the invaders.

### ii) Macrophages

If neutrophil killing does not suffice, the monocyte/macrophage arm of the innate response is activated. The ability of a macrophage to kill phagocytosed bacteria is influenced by

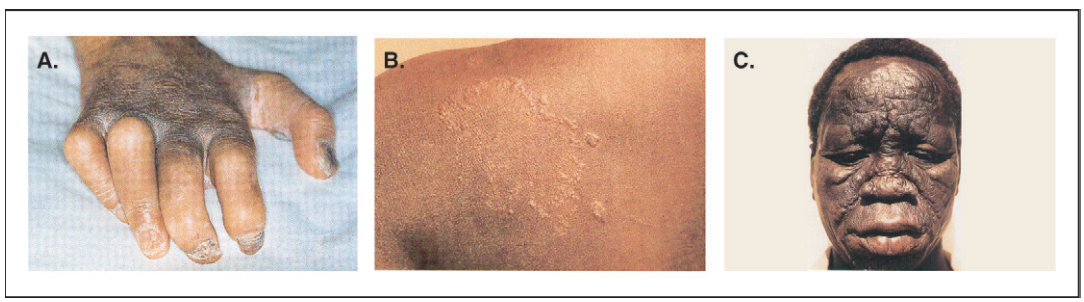



Plate 22-2

**Leprosy**
(A) Tuberculoid leprosy. (B) Leprosy characterized as the "middle of the spectrum." (C) Lepromatous leprosy. Reproduced with permission from Emond R.T.D., Welsby P.D., and Rowland H.A.K. (2003) "Colour Atlas of Infectious Disease," 4th edn. Elsevier Science.

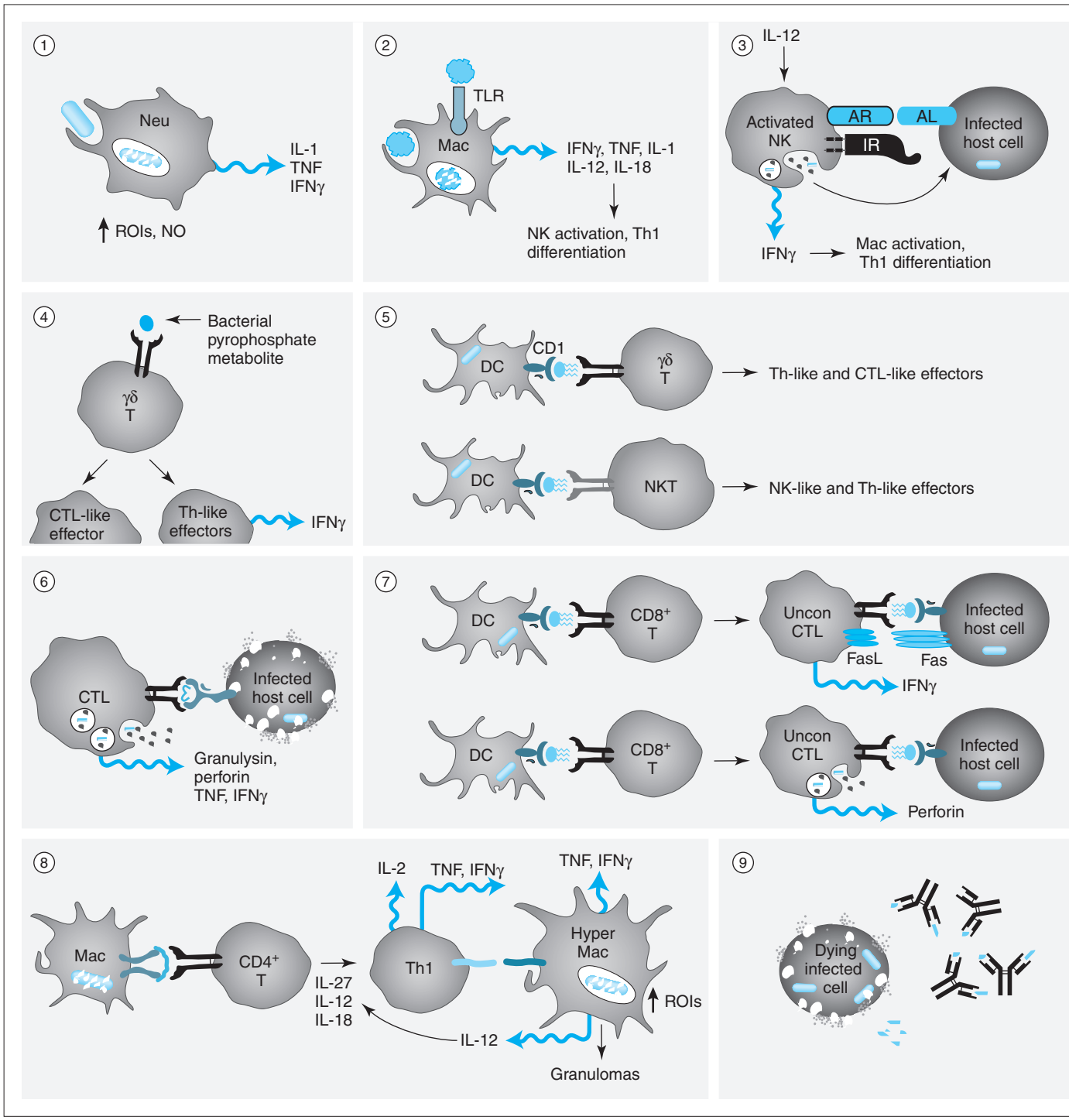



Figure 22-3

**Major Mechanisms of Immune Defense against Intracellular Pathogens**

(1) Phagocytosis of bacteria by neutrophils triggers phagosomal killing via respiratory burst and cytokine secretion. (2) Phagocytosis of bacteria by macrophages initiates phagosomal killing and secretion of cytokines that maintain inflammation, activate NK cells, and promote Th1 differentiation. TLR-mediated endocytosis also triggers cytokine secretion. (3) NK cells activated by IL-12 kill infected host cells by natural cytotoxicity and secrete IFNγ which activates macrophages and supports Th1 differentiation. (4) Phosphorylated metabolites released by a bacterium activate γδ T cells that generate T cell effectors. (5) Infected DCs present bacterial components on CD1 to γδ T cells and NKT cells. Once activated, these cells generate cytotoxic- and Th-like effectors. (6) CTLs recognizing bacterial peptides presented on MHC class I by an infected host cell kill the cell by perforin- and granzyme-mediated cytotoxicity. The CTL also secretes the anti-microbial molecule granulysin and pro-inflammatory cytokines. (7) Unconventional (Uncon) CTL subsets are activated by bacterial components presented on CD1 by infected DCs. One subset kills infected host cells by Fas killing, while another relies on perforin-mediated cytotoxicity. (8) Infected macrophages present bacterial peptides on MHC class II to $CD4^+$ T cells. In the presence of IL-27, IL-12, and IL-18, Th1 effectors differentiate and supply cytokines that both support the CTL response and hyperactivate macrophages. Hyperactivated macrophages produce increased levels of pro-inflammatory cytokines and ROIs that increase killing. Granuloma formation may occur if these measures are insufficient. (9) Bacterial components released from a dying infected cell can activate B cells to produce neutralizing antibodies. These antibodies intercept any bacterium temporarily transiting the extracellular environment.

proteins that scientists are only now beginning to investigate. For example, mice that have a mutation of a gene called Nramp1 (*natural resistance associated macrophage protein* 1) have an increased susceptibility to BCG infections. Upon phagocytosis of a microbe during the innate response, the wild-type Nramp1 protein is recruited to the membrane of the macrophage phagosome and integrates within it. It is thought that the presence of Nramp1 in the phagolysosomal membrane somehow influences the microenvironment within the phagolysosome and hence its killing ability. Human homologues of Nramp1 have been found in neutrophils, monocytes, and macrophages. Another group of proteins important for the killing of intracellular pathogens (at least in mice) are the *p47GTPases*, a family of GTP-hydrolyzing proteins of 47–48 kDa. These transmembrane enzymes, whose expression is greatly upregulated in response to IFNs and LPS, are thought to be localized in either the ER or the Golgi. Functionally, the p47GTPases appear to regulate the maturation and processing of pathogen-containing phagosomes, hastening the demise of the invader. Interestingly, different members of the p47GTPase family are required for effective resistance to different classes of intracellular pathogens. Studies of knockout mice have shown that, while the p47GTPase Igtp is required for normal resistance to the protozoan parasite *Leishmania major*, it does not play a crucial role in defense against the intracellular bacteria *L. monocytogenes* or *M. tuberculosis*. In contrast, knockout mice lacking the p47GTPase Lrg47 are highly susceptible to infection with *L. monocytogenes*, *S. typhimurium*, or *M. tuberculosis* as well as *L. major*.

As well as engulfment of pathogens by phagocytosis, TLR-mediated internalization by macrophages contributes to the resolution of intracellular bacterial infections. For example, lipoprotein and lipoglycan components of mycobacteria are recognized by TLR2 and TLR4 expressed on the surfaces of mammalian macrophages. Stimulation of these TLRs leads to NF-κB activation, which in turn leads to upregulation of inducible nitric oxide synthase (iNOS) and production of NO as well as the secretion of pro-inflammatory cytokines.

Despite the existence of Nramp1 and the p47GTPases, it is not unusual for an intracellular bacterium phagocytosed by activated macrophages to be resistant to routine phagosomal killing mechanisms. For this reason, macrophage hyperactivation is a crucial step in the clearance of many intracellular pathogens. The reader will recall that a positive feedback mechanism exists between macrophages and Th1 cells that is required for macrophage hyperactivation. Macrophages activated by exposure to the pathogen secrete IL-12, which influences activated T cells to differentiate into Th1 effectors. The IFNγ secreted by the activated Th1 cells acts on the macrophages to hyperactivate them such that they gain the enhanced microbicidal powers detailed in Chapter 4. Foremost among these powers is the capacity to produce the large quantities of ROIs and RNIs that efficiently kill almost all intracellular pathogens.

### iii) NK Cells

The IL-12 secreted by activated macrophages also activates NK cells. As described in Chapter 18, NK cells detect infected host cells by their lack of MHC class I expression (which is typically downregulated by the infection) and destroy them by natural cytotoxicity. Activated NK cells also secrete copious amounts of IFNγ, which promotes both macrophage hyperactivation and Th1 cell differentiation. Mice deficient for transcription factors such as STAT1 and IRF2 (which are required for IFNγ signaling) are highly susceptible to *Listeria* infection, reinforcing the key role IFNγ plays in fighting intracellular bacteria.

### iv) γδ T Cells

γδ T cells are also of importance in combating at least some intracellular infections. The reader will recall that the TCRs of the human Vγ2Vδ2 γδ T cell subset directly recognize small pyrophosphate-like molecules. Many species of intracellular bacteria (particularly the mycobacteria) release phosphorylated metabolites of this type as they attempt to colonize the host. These metabolites engage the Vγ2Vδ2 TCR, causing expansion of this lymphocyte population. High numbers of γδ T cells can be found in the acute lesions of leprosy patients, and in the lungs of mice immunized with aerosols containing mycobacterial components. As described in Chapter 18, it is thought that γδ T cell effector functions (cytolysis, IFNγ secretion) probably precede and overlap those of αβ T cells, filling any gaps in defense between the broadly specific neutrophils and NK cells, and the highly specific αβ T cells.

### v) $CD8^+$ T Cells

In terms of the adaptive immune response, defense mediated by MHC class I-restricted $CD8^+$ CTLs is critical in cases of infection by many species of intracellular bacteria. If the bacterium replicates in the cytosol of the infected cell, some of its component proteins will enter the endogenous antigen processing pathway and be presented on MHC class I on the host cell surface. As well, components derived from the degradation of a phagocytosed bacterium or antigens secreted by it can be released into the cytosol, allowing peptides from these molecules to be cross-presented on MHC class I. Such cells function as target cells for Tc in the local lymph node or memory CTLs in the periphery. For example, studies of *L. monocytogenes* have demonstrated that $CD8^+$ CTLs are crucial for resolving infections with this pathogen. Mice deficient for either αβ TCR components or β2m (and thus MHC class I) show decreased resistance to *L. monocytogenes* infection. Similarly, mice that are normally resistant to tuberculosis can be rendered susceptible if $CD8^+$ T cells are depleted by treatment with antibodies prior to infection. However, cytolysis via the perforin or Fas pathways does not appear to be a big factor in $CD8^+$ T cell-mediated defense against intracellular bacteria (unlike antiviral defense; see later). Studies in knockout mice have shown that Fas-mediated cytolysis is not required for any stage of host defense against *L. monocytogenes* or *M. tuberculosis*, and perforin-mediated cytotoxicity is important only for containing disease in the chronic stages of *L. monocytogenes* and *M. tuberculosis* infections. Rather, $CD8^+$ CTLs contribute to anti-bacterial defense primarily by releasing IFNγ and TNF, which are critical both for inflammatory cell influx and direct killing of infected cells, and by secreting *granulysin*, a molecule that has direct anti-microbial

activity. Knockout mice deficient for the expression of either TNF or IFNγ or the receptors for these molecules show increased susceptibility to infections with *L. monocytogenes*. Furthermore, animals treated with anti-TNF or anti-IFNγ antibodies show increased susceptibility to listeriosis, while those treated with anti-IL-4 actually show increased resistance. Human patients lacking the IFNγ receptor are highly susceptible to certain types of mycobacterial infections.

### vi) Unconventional CD8$^+$ CTLs

Unconventional CTL subsets also contribute to defense against intracellular bacteria. Many types of intracellular bacteria secrete proteins that can be processed into peptides containing N-formyl-methionine. As we learned in Chapters 10 and 11, these peptides can be bound by non-classical MHC class Ib molecules (such as H-2M3) and presented to αβ CD8$^+$ T cells. Several *Listeria* peptides can be presented by H-2M3 in mice, and a role for this type of presentation has been proposed for the early stages of the response to *Listeria* infection. However, this type of priming appears to induce only minimal memory responses. In the case of mycobacteria, infected DCs can present non-peptide glycolipid bacterial antigens on the non-polymorphic MHC-like molecule CD1b. Two subsets of CD1b-restricted CTLs have been defined in *M. tuberculosis*-infected mice. One subset uses Fas-mediated cytolysis to kill infected host cells (but not the pathogen), while the other subset uses perforin-mediated cytolysis to simultaneously eliminate both the infected host cell and the pathogen. Both subsets also secrete IFNγ. In humans, CTLs directed against glycolipid antigens have been isolated from tuberculosis patients, and these cells directly recognize and lyse infected macrophages *in vitro*. γδ T cells and NKT cells bearing CD1c- and CD1d-restricted TCRs may also play a role in anti-mycobacterial defense *in vivo* but the antigens recognized by these TCRs have yet to be identified.

### vii) CD4$^+$ T Cells

CD4$^+$ T cells make a significant contribution to defense against intracellular bacteria not only because of the IL-2 they secrete to support Tc differentiation but also because these cells are required for the hyperactivation of macrophages. Bacterial antigens either secreted by the bacteria themselves or released by necrotic infected cells are taken up by professional APCs and presented to CD4$^+$ T cells in association with MHC class II. The IFNγ produced by NK cells activated early in the infection favors the differentiation of Th1 effectors. Activated Th1 cells then supply the intercellular contacts (particularly CD40L) and TNF and IFNγ that drive the hyperactivation of macrophages and, if necessary, the formation of granulomas (see later).

While the IL-12 secreted by activated and hyperactivated macrophages and DCs is vital for the Th1 response to intracellular pathogens, it is not the only important cytokine. As discussed in Chapter 17, IL-18 acts synergistically with IL-12 to promote IFNγ production and Th1 differentiation. Resistance to infection by species of *Yersinia*, *Salmonella*, and *Cryptococcus* is enhanced by administration of IL-18 to wild-type mice, and IL-18$^{-/-}$ mice show increased susceptibility to infection with intracellular pathogens such as mycobacteria and *L. major*. IL-27 is required for the initiation (but not maintenance) of Th1 responses. Mice lacking IL-27R thus show decreased IFNγ production, which increases their susceptibility to *L. major* infection.

The importance of the Th1 response in defense against intracellular pathogens is nicely illustrated in human immune responses to *M. leprae* infection. When the T cells surrounding the lesions of the mildly affected tuberculoid leprosy patients described previously were isolated and tested for cytokine production, they were found to secrete IFNγ. It was hypothesized that these patients mounted a Th1 response that promoted cell-mediated immunity and ameliorated the disease. In contrast, the T cells surrounding the lesions in the more severely affected lepromatous patients were found to produce predominantly IL-4 and IL-10. These patients appeared to have mounted a Th2 response that favored humoral immunity (less effective against intracellular pathogens) over cell-mediated immunity. This difference is likely attributable to genetic differences between patients that affect the Th1/Th2 polarization of Th cells responding to this pathogen.

### viii) Granuloma Formation

When an intracellular pathogen is able to resist killing even by CTLs and hyperactivated macrophages, the body takes the approach of "If you can't remove 'em, confine 'em." A group of hyperactivated macrophages fuses to form a granuloma that walls off the pathogen from the rest of the body (as described previously for chronic TB infection) (Fig. 22-4 and Plate 22-3). Scattered among the macrophages in the inner layer of the granuloma are numerous CD4$^+$ T cells, while an outer ring of CD8$^+$ T cells forms the exterior layer. Eventually the exterior of the granuloma becomes calcified and fibrotic, and cells in the center undergo necrosis. In some cases, all the pathogens trapped in the dying cells are killed and the infection and inflammation are resolved. In other cases, a few pathogens remain viable but dormant within the granuloma, causing it to persist. Granuloma persistence is an overt sign that the disease is becoming chronic. An event that results in the breakdown of the structure of the granuloma can release the trapped pathogens back into the body to commence replication anew. Should the host be immunosuppressed for some reason and unable to marshal the T cells and macrophages necessary to form new granulomas, the pathogen may be released into the bloodstream, from which it can infect other organs throughout the body and even cause death.

Cytokines play a critical role in granuloma formation. Sustained IFNγ production by Th1 cells and CTLs is required to maintain macrophage hyperactivation. TNF production by hyperactivated macrophages is crucial not only for early chemokine synthesis (to recruit fresh cells to the incipient granuloma) but also for aggregating these cells and establishing the "wall" around the invaders. Secretion of IL-4 and IL-10 by Th2 cells late in the adaptive response serves to control the formation of granulomas, damping them down as the bacterial threat is contained.

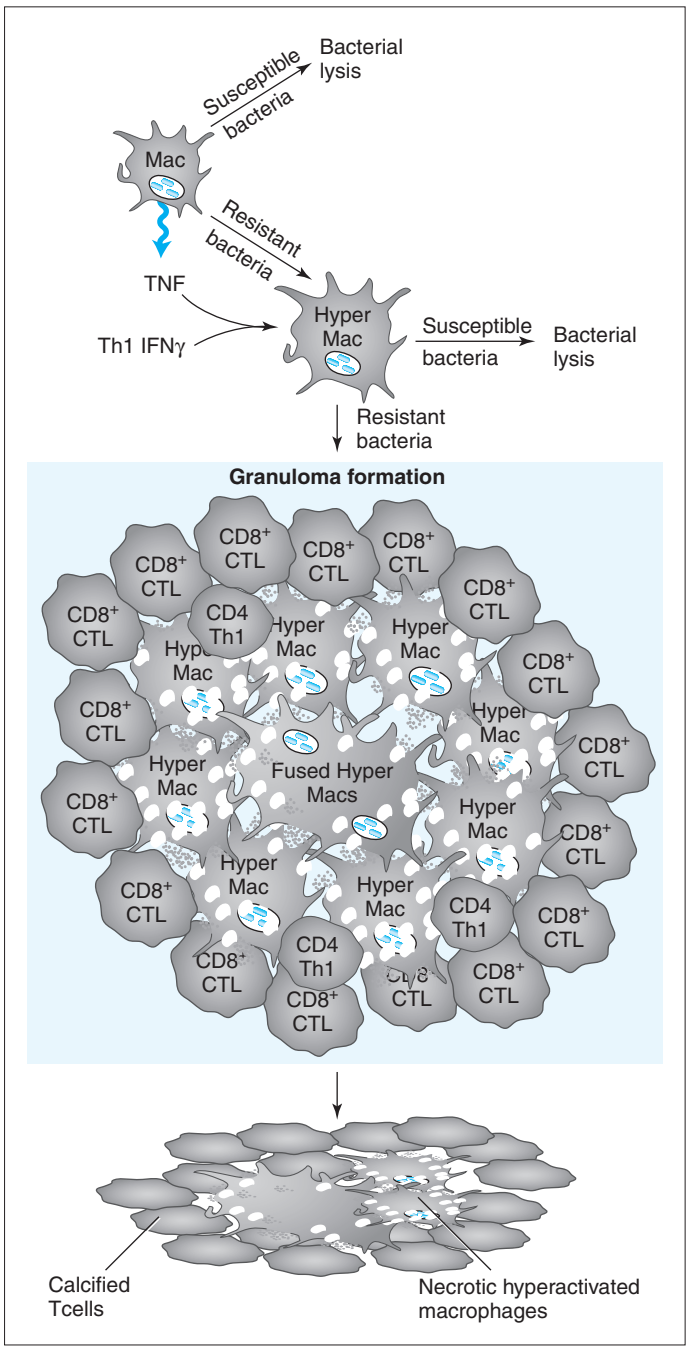



Figure 22-4

**Granuloma Formation**
Most bacteria engulfed by activated macrophages succumb to phagosomal killing in these cells. However, bacteria resistant to this mode of destruction induce the macrophage to increase its secretion of TNF. In the presence of this TNF plus IFNγ produced by activated Th1 cells, the macrophage becomes hyperactivated and capable of killing most initially resistant bacteria due to its increased production of ROIs and RNIs. However, if any bacteria survive, the hyperactivated macrophages fuse to form the center of a granuloma. Surrounding the fused macrophages are additional hyperactivated macrophages and activated $CD4^+$ and $CD8^+$ T cells. Eventually, the center of the TNF-permeated granuloma becomes necrotic, killing most bacteria, and the surrounding T cells calcify to physically wall off any bacteria surviving this assault.

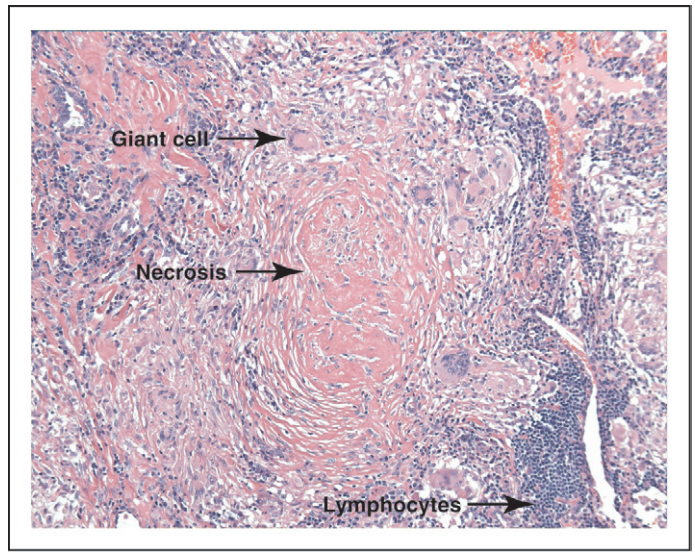



Plate 22-3

**Granuloma**
A central zone of necrosis is surrounded by activated macrophages and multinucleated giant cells (fused macrophages), with a rim of fibrosis and lymphocytes. Courtesy of Dr. David Hwang, Department of Pathology, University Health Network, Toronto General Hospital.

### ix) Humoral Defense

Although it may seem counterintuitive at first, antibodies can contribute to defense against intracellular pathogens. Some antibodies have been shown to block the access of bacteria to host receptors used for cell entry. In addition, newly arrived bacterial cells or those transiting the extracellular space before invading the neighboring host cell can be opsonized or subjected to classical complement-mediated lysis. It is further speculated that antibodies taken into the phagosome with the bacterium can act in this location. For example, monoclonal antibodies to *listeriolysin O* (LLO), a bacterially produced molecule that facilitates the escape of *L. monocytogenes* from the phagosome into the host cytosol, have been shown to provide protection against *L. monocytogenes* infections in this way.

## III. EVASION STRATEGIES

Strategies used by intracellular bacteria to evade the immune response are summarized in Table 22-7.

### i) Avoiding Phagolysosomal Destruction

Evolution has confined intracellular pathogen killing to the phagosome of professional phagocytes. Consequently, internal damage to the phagocyte itself is prevented, and non-phagocytic cells (which do not create phagosomes) run no risk at all. In view of the compartmentalized nature of phagocytosis, intracellular bacteria can evolve in one of two ways to improve their chances of survival. They can either enter and replicate in non-phagocytic cells, or enter phagocytic cells and employ strategies to avoid phagolysosomal destruction. One example of an intracellular bacterium that targets a non-phagocytic cell is

Table 22-7 **Evasion of the Immune System by Intracellular Bacteria**

| Immune System Element Thwarted | Bacterial Mechanism |
|---|---|
| Phagolysosomal destruction | Reproduce in non-phagocytes<br>Synthesize proteins blocking lysosomal fusion and/or phagosomal killing<br>Recruit host proteins blocking lysosome function<br>Reverse acidification of phagosome |
| Microbicidal action and hyperactivation of macrophages | Block intracellular signaling leading to expression of microbicidal genes and IFN-induced genes<br>Produce phenolic glycolipid to neutralize ROI<br>Produce enzymes breaking down ROI and $H_2O_2$ |
| Antibodies | Spread to new host cell via pseudopod invasion |
| T cells | Anergize T cells via contact with LLO protein |
| APCs | Downregulate CD1 expression<br>Block maturation or migration of DCs |

*M. leprae*, which infects Schwann cells of the peripheral nervous system. The Schwann cells, which wrap around the axons of nerves and generate the myelin sheath, express a surface protein called α-*dystroglycan*. This protein normally has a role in early development and morphogenesis, but in this case acts as a receptor for *M. leprae*. The microbe enters the Schwann cells and is protected from phagocytosis. Similarly, *L. monocytogenes* expresses *internalin* proteins that bind to the host adhesion molecule E-cadherin, allowing the bacteria to enter non-phagocytes. *L. monocytogenes* also deliberately accesses phagocytes using the host cell Fc and complement receptors, but then employs survival tactics to prevent killing within the phagosome. These tactics include the synthesis of the listeriolysin O protein mentioned previously. LLO induces pore formation in the membrane of the phagolysosome, allowing the bacterium to escape into the relative safety of the cytoplasm.

*M. tuberculosis* has devised several different ways of avoiding phagolysosomal destruction. First, when this bacterium finds itself being engulfed in a macrophage phagosome, it recruits a host protein called TACO (tryptophan-aspartate-containing coat protein) to the cytoplasmic surface of the developing phagosome. The presence of TACO inhibits the fusion of the phagosome with lysosomes and other vesicles containing hydrolytic enzymes and specialized killing components. Secondly, mycobacteria also produce $NH_4^+$, which reverses the acidification of phagolysosomes and promotes fusion with harmless endosomes. Thirdly, *M. tuberculosis* infection of macrophages interferes with STAT1-mediated transcriptional responses to IFNγ signaling, blocking the expression of genes needed for microbicidal action and hyperactivation. As a result of these three strategies, mycobacteria can survive within host phagosomes for long periods. The bacteria may remain active, or enter a state of dormancy in which many of their metabolic pathways are downregulated.

Other intracellular bacteria focus on blocking the ROIs produced within the phagosome. The phenolic glycolipid found in *M. leprae* neutralizes ROIs, while other bacterial species produce superoxide dismutase and catalase that break down ROIs and hydrogen peroxide. Catalase may also inhibit the generation of RNIs.

*S. typhi* and *S. typhimurium* preferentially enter the body via the M cells of the PPs. Once in the dome below the FAE, these bacteria induce resident macrophages to capture them by macropinocytosis. Inside the macrophages, the bacteria secrete a protein called SpiC that efficiently blocks fusion of lysosomes with phagosomes, and other molecules that decrease the recruitment of NADPH oxidase to the phagolysosome. Still other bacterial products confer resistance to cationic peptides. These measures permit the *Salmonella* to survive within the macrophage for a relatively short period of time. However, because macrophages are mobile, the bacterial infection is quickly disseminated all over the body. In typhoid carriers, the disease becomes chronic, as the bacteria accumulate in the gall bladder and are shed for several months or even indefinitely into the feces. Transmission to fresh hosts is achieved under conditions of poor sanitation when the feces of an infected person enter the water supply consumed by non-infected individuals. An infected human does make antibodies to the capsule of *S. typhi*, and carriers have such antibodies in their plasma, but the antibody is not protective while the bacterium is hidden in the macrophages. Eventually, macrophages harboring *S. typhi* are induced to undergo apoptosis.

### ii) Avoiding Antibodies

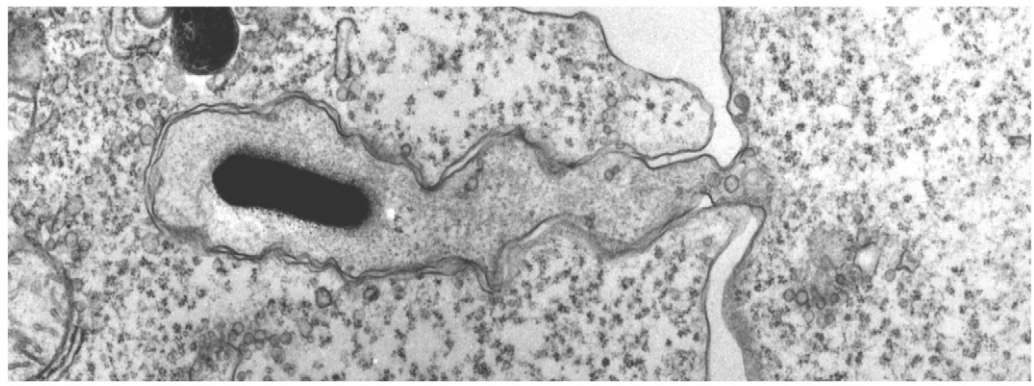
The ultimate escape for an intracellular pathogen is to make it into a new host cell without attracting the attention of the immune system at all. Ultrastructural studies have shown that, in host cells infected with *L. monocytogenes*, the bacterium induces the actin-based formation of a pseudopod that invaginates into a neighboring non-phagocytic cell. The neighboring cell engulfs the pseudopod (which contains the bacterium plus bits of the original host cell cytoplasm) and pinches it off to form a secondary vacuole surrounded by a double plasma membrane (Plate 22-4). The bacterium then uses LLO and phospholipases to forge its way through both sets of host membranes and enters the cytoplasm of the new host cell, free to start the cycle anew. The beauty of this tactic is that the bacterium is never extracellular, meaning that antibodies can never bind to it. Indeed, although low levels of natural IgM antibodies to *Listeria* have been detected in naive inbred mice, these antibodies do not play a significant role in resistance to natural *Listeria* infections.

### iii) Avoiding T Cells

The LLO molecule of *L. monocytogenes* provides another means of defense in addition to its pore-forming capacity. It has been observed that contact with LLO irreversibly inactivates $CD4^+$ T cells. In *in vitro* experiments, antigen-specific $CD4^+$ T cells were exposed to APCs that were either infected with *L. monocytogenes* or treated with bacterial LLO peptides. T cells treated in this way became anergic and could not be activated by subsequent exposure to a known stimulatory

Plate 22-4

**Pseudopod Invasion**
A pseudopod containing a *Listeria monocytogenes* bacterium is extended by an infected cell (right) and engulfed by an uninfected neighboring cell (left), allowing the bacterium to spread without exposure to host antibody. Courtesy of Daniel Portnoy and Lewis Tilney, University of California, Berkeley.



peptide, even after LLO was removed. The mechanism underlying this inactivation is unknown but may be related to the antagonism of TCRs by altered peptide ligands (see Ch.14).

*M. tuberculosis* escapes T cell attack by interfering with APC function. Infection of DCs by this pathogen promotes downregulation of the expression of MHC class I and II and CD1, decreasing the presentation of non-peptide antigens. The number of T cell clones that can be brought to bear on the pathogen is thus reduced.

## D. Immunity to Viruses

### I. WHAT ARE VIRUSES?

#### i) General Characteristics

Like many species of bacteria, viruses are obligate intracellular pathogens. However, unlike bacteria, viruses consist only of DNA or RNA genomes packaged in proteinaceous capsids. Viruses therefore do not have their own protein synthesis machinery and rely on subverting that of the host cell. The genomes of viruses are much smaller than those of other classes of pathogens, being limited to about 100 genes at most; the smallest viruses make do with four genes. Most of the genes in a virus genome encode structural proteins or are required for viral replication, but a surprisingly large number of genes in the more complex viruses encode proteins that undermine the host immune response or regulate host cell apoptosis. Many of these genes are dispensable for viral replication *in vitro*, emphasizing their function as countermeasures to the host immune response *in vivo*. Viruses with a small genome count on rapid replication and dissemination to new host cells to establish an infection before the immune system can respond. Viruses with larger genomes need more time to replicate and are transmitted more slowly; accordingly, these organisms have developed ways of interfering with various components of the host immune response that allow sufficient time for the establishment of an infection.

Viruses usually use a host receptor to bind to and enter the cell, followed by replication and virion assembly within the cell. Progeny virions released into the local area can infect neighboring host cells and initiate new replicative cycles that facilitate widespread dissemination of the virus. Progeny virions that reach the blood circulation are free to spread systemically. Different viruses have different relationships with their hosts. Viruses may vary with respect to the species they can infect (host range), the kinetics of their replication, and their propensity to cause either acute or chronic disease. Naturally, a successful virus is not so virulent that it wipes out all members of its target species, since that would prevent the virus's own future replication. Viruses that damage the host cell during infection are said to be *cytopathic*, while those that merely take over cell functions without damaging the host cell are *non-cytopathic*. In addition to virally induced cytopathy, it is not unusual for the host's own immune response to the virus to result in immunopathic damage to host tissues.

After an initial infection, some types of viruses remain in the body and establish a persistent infection. Unlike bacteria and parasites, which often give rise to chronic disease in cases of persistent infection, most viruses that persist in the body do so in a *latent* state. During latency, viral activity is held in check by cell-mediated immunity. The host experiences no symptoms and the virus remains in a non-infectious mode in which no new virus particles are assembled. However, as the host ages or faces immune system challenges such as other infectious agents, cancer, or immunosuppressive drug treatments, the cell-mediated response weakens. This weakening increases the likelihood that the latent virus will become reactivated, leading to recommencement of a productive infection with associated symptoms. A common example of this type of latent viral infection occurs with the virus that causes chicken pox, *varicella zoster virus* (VZV; also known as human herpesvirus 3, or herpes zoster). After an initial infection that results in the characteristic chicken pox rash, the virus moves up to the dorsal root ganglia of sensory nerves that reach into the skin and becomes latent, usually for years. Upon reactivation, the viral infection spreads down the affected nerves to the skin, causing the formation of vesicles called "shingles" that are very painful to the host and are teeming with infectious virus particles. *Herpes simplex virus* (HSV) acts in a similar way on nerves supplying the mouth

area, giving rise to cold sores after a period of latency. *Cytomegalovirus* (CMV) persists in a latent infection that causes no symptoms and is not infectious as long as cell-mediated responses are intact and ongoing. If the virus becomes reactivated, patients may come down with pneumonitis or hepatitis and may shed infectious virus in their urine.

The oncogenic viruses are an important group of latent viruses. Both DNA and RNA viruses are represented in this heterogeneous group, their common characteristic being an association with malignancy in a small percentage of hosts. Infection with these viruses is usually inconsequential to everyday health, but, in some individuals, the replication of these pathogens disturbs the host cell cycle in such a way that the cell is transformed and becomes cancerous. The DNA oncogenic viruses include *human papillomavirus* (HPV; cervical cancer), *Epstein-Barr virus* (EBV; Burkitt's lymphoma and nasopharyngeal cancer), and *hepatitis B virus* (HepB; liver cancer). The RNA oncogenic viruses are all retroviruses, meaning that they possess a reverse transcriptase enzyme that allows them to make a cDNA copy of their RNA genome that can integrate into the host cell DNA. In humans, the retrovirus *human T cell leukemia virus* (HTLV) causes T cell leukemias and lymphomas (see Ch.30). The *human immunodeficiency virus* (HIV), which causes AIDS, is also a retrovirus (see Ch.25). HIV-infected individuals have a higher incidence of various lymphomas (see Ch.30). More on oncogenic viruses and immune responses to them appears in Chapter 26.

### ii) Characteristics of Selected Viruses

Table 22-8 contains an overview description of important virus families. Table 22-9 contains a summary of illnesses caused by pathogenic viruses mentioned in this chapter. More detailed information on several well-studied and important human viruses appears in the following sub-sections. Additional viral human pathogens are discussed in Chapter 23 in the context of vaccination.

**iia) Adenovirus.** Adenovirus is a DNA virus that most often infects the upper respiratory tract of young children but that can also affect the eye and gastrointestinal tract. Disease is

Table 22-8 **Overview of Virus Families**

| Virus Family | Envelope | Size (nm) | Important Members |
|---|---|---|---|
| **DOUBLE-STRANDED DNA** | | | |
| Adenoviridae | No | 70–90 | Adenoviruses |
| Hepadnaviridae | Yes | 42 | HepB |
| Herpesviridae | Yes | 150–200 | EBV, varicella, KSHV, HSV, CMV |
| Papovaviridae | No | 40–57 | Papillomavirus, polyoma, simian virus |
| Poxviridae | Yes | 200–350 | Vaccinia, variola, molluscum contagiosum |
| **SINGLE-STRANDED RNA** | | | |
| Arenaviridae | Yes | 110–130 | LCMV |
| Bunyaviridae | Yes | 90–120 | Hantavirus |
| Coronaviridae | Yes | 80–160 | Coronavirus, SARS virus |
| Filoviridae | Yes | 80–1,400 | Ebola virus, Marburg virus |
| Flaviviridae | Yes | 40–50 | HepC, yellow fever, dengue fever, West Nile virus |
| Paramyxoviridae | Yes | 150–300 | Parainfluenza virus, mumps, measles |
| Picornaviridae | No | 28–30 | HepA, enterovirus, rhinovirus, polio virus, Coxsackie virus |
| Rhabdoviridae | Yes | 70–180 | VSV, rabies virus |
| Togaviridae | Yes | 60–70 | Alphaviruses, rubella |
| **SINGLE-STRANDED RNA IN SEGMENTS** | | | |
| Orthomyxoviridae | Yes | 80–200 | Influenza viruses |
| **DOUBLE-STRANDED RNA** | | | |
| Reoviridae | No | 60–80 | Reovirus, rotavirus |
| Retroviridae | Yes | 100–120 | Lentiviruses (HIV), HTLV |

Table 22-9 **Examples of Viruses and the Diseases They Cause**

| Virus | Disease |
|---|---|
| Adenovirus | Acute respiratory infections |
| Epstein-Barr virus (EBV) | Infectious mononucleosis, Burkitt's lymphoma, nasopharyngeal cancer |
| Hepatitis A virus (HepA or HAV) | Hepatitis, cirrhosis, liver cancer |
| Hepatitis B virus (HepB or HBV) | Hepatitis, cirrhosis, liver cancer |
| Hepatitis C virus (HepC or HCV) | Hepatitis, cirrhosis, liver cancer |
| Herpes simplex (HSV) | Cold sores |
| Human cytomegalovirus (huCMV or HCMV) | Pneumonitis, hepatitis |
| Human immunodeficiency virus (HIV) | AIDS, lymphomas |
| Human papillomavirus (HPV) | Skin warts, asymptomatic genital infections, cervical cancer |
| Human T cell leukemia virus (HTLV) | T cell leukemias and lymphomas, chronic inflammation of the spinal cord |
| Influenza virus | Influenza (respiratory infection; often called the "flu") |
| Kaposi's sarcoma herpesvirus (KSHV) | Kaposi's sarcoma (associated with AIDS), B lymphoma |
| Lymphocytic choriomeningitis virus (LCMV) | Brain damage in mice, loss of motor control; viral meningitis in severe human infections |
| Measles virus (MV) | Measles |
| Molluscum contagiosum (MCV) | Benign skin tumors in humans |
| Mouse cytomegalovirus (muCMV or MCMV) | Pneumonitis, hepatitis in mice |
| Mouse mammary tumor virus (MMTV) | Mammary tumors in mice |
| Polio virus | Poliomyelitis, post-polio fatigue |
| Rabies virus | Rabies |
| Rhinovirus | Common cold |
| SARS virus | Severe acute respiratory syndrome |
| Varicella zoster virus (VZV) | Chicken pox, shingles (herpes zoster) |
| Variola virus | Smallpox |
| Vesicular stomatitis virus (VSV) | Mouth lesions and hoof loss in horses; flu-like symptoms in humans |
| West Nile virus (WNV) | Flu-like illness, encephalitis, fatigue; often fatal in horses and some species of birds |

usually acute but mild, with symptoms ranging from those associated with the common cold to tonsillitis. If the virus attacks the lower respiratory tract, bronchitis and pneumonia can result. While 50% of adenovirus infections in otherwise healthy children are asymptomatic, adenovirus can kill immunocompromised individuals.

Adenovirus is one of the best-studied pathogens at the molecular level. The virus first expresses E1A genes that force the infected host epithelial cell to enter S phase. The viral DNA then replicates after about 7 hours. Various E1B, E2, E3, and E4 transcription units are subsequently activated that supply structural proteins for the virus and other proteins that control host cell functions and viral resistance to the immune response. Virions are assembled within 24 hours and are released from the lysed host cell by 48–72 hours.

**iib) Cytomegalovirus.** Cytomegaloviruses are β-herpesviruses, meaning that they are large, double-stranded DNA viruses. Human CMV is endemic in all human populations but causes only sub-clinical infections in immunocompetent hosts due to control by $CD8^+$ T cells. However, in immunocompromised hosts, such as fetuses, AIDS patients, and transplant recipients (who may acquire CMV from their transplants), the active virus can cause severe or even fatal disease. About 10–15% of CMV-infected fetuses suffer brain damage, while infected adults may come down with pneumonia, hepatitis, nephritis, encephalitis, and/or an increased incidence of bacterial and fungal infections. CMV is also capable of adopting a latent state (see later) in which the inactive virus is found predominantly in the kidney, liver, and heart.

**iic) Epstein-Barr virus.** EBV is a DNA herpesvirus that preferentially infects B cells of young children by exploiting host CR2 molecules. In developing countries, EBV is endemic and almost all young children contract a mild infection. This early exposure confers a degree of resistance to EBV in later life, and adolescents and young adults are rarely ill with EBV infections. In developed countries, early childhood EBV infection is much less common, and an individual's first encounter with the virus may occur during adolescence. Such later infections produce a more serious glandular fever called *infectious mononucleosis* ("mono"), which is characterized by extreme fatigue. This type of EBV infection is particularly contagious, in part because the virus is very active in the salivary gland ("kissing disease"). In addition, as mentioned previously, EBV is an oncogenic virus. It persists in the body and may cause malignant transformation of cells in a small proportion of cases. Persistent EBV infection of tonsillar cells can result in nasopharyngeal cancer, while persistence of EBV in resting memory B cells leads to Burkitt's lymphoma. In the laboratory, EBV is often used to immortalize cultured B cells *in vitro* to establish B cell lines.

**iid) Hepatitis B virus.** The hepatitis B virus is an enveloped DNA virus that preferentially infects hepatocytes. Clinical symptoms of HepB infection include jaundice, nausea, vomiting, fatigue, and pain in the abdomen and joints. HepB infections may be either acute or chronic. Transmission is primarily by sexual contact or via contaminated needles used to inject intravenous drugs, although body fluids such as saliva and breast milk can be infectious as well. About 30% of acute infections are asymptomatic. When the virus adopts a latent state in which the viral genome integrates into the host genome, a chronic HepB infection is established and these persons become HepB carriers (who may or may not show symptoms). Chronic HepB infections may eventually lead to more serious conditions such as cirrhosis of the liver and hepatic cancer. The propensity for HepB infection to become chronic is greatest in the very young. That is, in 90% of individuals who are infected with the HepB virus when under the age of 1 year, the infection becomes chronic. In contrast, only 5% of those who are infected when over the age of 5 years continue to harbor the virus latently. The WHO estimates that, worldwide, 1.2 billion people are infected with HepB, and 350 million of these infections are chronic. About 15–25% of chronically infected persons die of HepB-related disease each year.

**iie) Hepatitis C virus.** The hepatitis C virus is a small, enveloped, single-stranded RNA virus with effects very similar to those of HepB, except that the infection becomes chronic at a much higher rate: fully 80% of all individuals infected are unable to clear the virus. The major mode of HepC transmission is via exposure to contaminated blood, either through blood transfusion (see Box 25-1 in Ch.25) or the sharing of contaminated needles. It remains controversial whether HepC is also transmissible through sexual contact. Worldwide, at least 3–4 million new HepC infections occur each year, and an estimated 3% of the world's population is currently infected. Of those chronically infected, 10–20% go on to develop cirrhosis of the liver or liver cancer, and an estimated 0.4% of these die each year. In the United States, chronic liver disease is the 10th leading cause of death among adults, and studies suggest that 40% of all such cases are due to HepC infection. In developed countries, chronic HepC infection is the most common reason for liver transplants.

The mechanism by which HepC establishes chronic infection is not yet understood. Most individuals attacked by HepC mount humoral and cell-mediated responses against the virus, but these responses are not usually sufficient to clear it. Moreover, HepC exhibits considerable genetic heterogeneity, with six major genotypes and over 100 strains. The envelope proteins of different HepC strains can diverge by as much as 50%, such that neutralizing antibodies that are effective against one strain may not be effective against another strain. More on the difficulties of dealing with HepC appears in Chapter 23.

**iif) Human immunodeficiency virus (HIV).** Because an extensive discussion of HIV and AIDS appears in Chapter 25, we include only a few brief comments here. HIV is a retrovirus that exhibits extreme *antigenic variation*. HIV infects both macrophages and $CD4^+$ T cells and destroys the latter. The lack of $CD4^+$ T cells fatally cripples adaptive immune responses in these patients, and they usually die of either opportunistic infections or unusual tumors. The WHO estimates that, worldwide, over 42 million people are currently infected with HIV and that more than 3 million infected persons die each year of AIDS.

**iig) Influenza virus.** The influenza virus is an enveloped RNA virus with a segmented genome. As most of us know from personal experience, the "flu" is a nasty respiratory infection that initiates in ciliated epithelial cells but eventually produces symptoms that affect the entire body. Much of the malaise associated with the flu can be attributed to the large quantities of IFNα produced by immune system cells fighting the infection. Headaches, chills, high fever, muscle aches, weakness, and dry cough are common symptoms, most of which are resolved within 2 weeks. However, the virus abrogates the normal function of the ciliated epithelial cells lining the respiratory tract, decreasing the expulsion of pathogens in the mucus. Macrophage and neutrophil functions may also be suppressed in the local area of infection. As a result, opportunistic bacteria may become established in the respiratory tract and go on to cause potentially fatal pneumonia.

Part of the difficulty in combating influenza viruses is that a number of different strains exist in the human population. Because the principal proteins of these strains differ slightly (another example of antigenic variation), an immune response mounted against one strain does not guarantee protection against another strain. Thankfully, neither chronic nor latent infections occur with influenza virus. Influenza virus has been responsible in the past for several hemispheric and global epidemics in which the elderly and the immunocompromised were killed in large numbers. Even today, influenza virus infections cause significant morbidity and mortality, particularly among the aged and chronically ill. On a global scale, it is estimated that the annual bout of influenza infection causes 3–5 million cases of severe disease and 250,000–500,000 deaths.

**iih) Measles virus.** The measles virus is a cytopathic, enveloped RNA virus that initially attacks the upper respiratory tract but then spreads via the lymphatics and blood circulation to most other tissues. The characteristic red "measles spots" that appear all over the body are due to cell-mediated immune responses against infected host cells. Acute measles infections are highly contagious and the virus is easily spread to new hosts through respiratory secretions. Moreover, measles virus can survive for at least 60 minutes in aerosolized droplets, allowing it to spread through ventilation systems. Globally, an estimated 40 million new measles infections occur each year.

At the cellular level, the virus primarily targets monocytes, macrophages, lymphocytes, and DCs by binding to a membrane glycoprotein called SLAM (*signaling lymphocyte activation molecule*; CD150). However, the measles virus can also penetrate the walls of cerebral capillaries and enter the CNS, where it infects neurons. Due to the loss of function of multiple types of immune system cells, measles

patients experience profound immunosuppression that makes them highly susceptible to secondary infections that may be severe or even fatal. In unvaccinated individuals, the disease can have significant mortality: 1–2 million deaths due to measles occur worldwide each year. While most survivors enjoy lifelong immunity to this virus, they are not entirely without risk. Some variant strains of the measles virus have defects in their surface proteins that block expression of viral antigens on the infected host cell surface, meaning that these cells escape the immune response. In a small percentage of individuals, these variants persistently infect the CNS and cause a chronic, progressive neurological disease called *subacute sclerosing panencephalitis* (SSPE). More on the measles virus appears in Chapter 23.

**iii) Polio virus.** Polio virus is a small, non-enveloped RNA virus that replicates first in the intestinal tract. The virus is shed into the feces of an infected individual and spreads to new hosts via feces-contaminated food or water. In the later stages of an infection, the virus moves to the blood and spreads to the spinal cord and CNS. While most infected individuals recover completely, in a small number of patients the virus causes *poliomyelitis*, permanent muscle paralysis due to the destruction of the motor nerves of the CNS. In fact, in the pre-vaccine era, poliomyelitis was the leading cause of permanent disability in the United States. The disease was called "infantile paralysis" at that time because of the large number of children affected. When viral destruction of brain stem cells resulted in paralysis of the respiratory muscles, the patient had to be placed in an "iron lung" (artificial breathing apparatus). Even today, polio virus infection is fatal in 2–5% of poliomyelitic children and in 15–30% of adults. In aging survivors, the neurological damage suffered during an acute infection may be manifested as "post-polio fatigue," similar in symptoms to chronic fatigue syndrome. Polio is now almost unknown in the Americas, Europe, and the Antipodes due to a very successful vaccination campaign (see Ch.23), although it continues to plague some populations in Asia and Africa.

**iij) Rabies virus.** *Rabies* is a disease characterized by hyperexcitability and spasms of the mouth and pharynx that occur when swallowing liquids. This latter feature can sometimes lead to "foaming at the mouth" and/or a fear of water ("hydrophobia"). The disease is caused by the rabies virus, an enveloped RNA virus. Upon entering the body, the virus replicates first in skeletal muscle and connective tissue. It then enters and spreads along the peripheral nerves to the spinal cord and CNS, where it eventually causes encephalitis. Initial symptoms are slow to develop, and may include irritability, fever, pain, and fatigue that can progress to hallucination and paralysis. The rabies virus readily infects cats, dogs, raccoons, foxes, bats, and skunks, all of which are creatures that interact quite frequently with humans. The bite of a rabid animal is usually fatal for humans unless a course of injections of protective anti-rabies antibodies is undertaken immediately (see Ch.23). The virus replicates in slow waves, meaning that the passive supply of relatively short-lived antibodies must be replenished several times to ensure elimination of the virus.

**iik) Rhinovirus.** Rhinovirus (rhino, "nose") is a non-enveloped, single-stranded RNA virus and a member of the picornavirus family. Over 100 rhinoviruses are known. About 50% of common colds are caused by some kind of rhinovirus. (About 10% of common colds are due to infection with a different type of virus, such as adenovirus, while the cause in 40% of cases is unknown.) Rhinoviruses thrive in the upper respiratory tract, particularly the nose and throat. The infection is thus characterized by the familiar symptoms of sneezing, excessive nasal secretion ("runny nose"), and congestion ("stuffy nose"). Complications of rhinovirus infection include spreading of the virus from the throat to the sinuses, lower respiratory tract, and middle ear, resulting in sinusitis, laryngitis, and otitis media, respectively.

**iil) SARS virus.** The virus that causes SARS (*severe acute respiratory syndrome*) is a novel member of the coronavirus family. Coronaviruses are enveloped, single-stranded RNA viruses with an asymmetric shape and surface spikes positioned so as to resemble a crown. Most human coronaviruses are associated with only mild respiratory disease. Thus, the world was caught off-guard in early 2003 by the severity of the atypical pneumonia caused by the newly emerged SARS coronavirus. Over 8000 cases of infection leading to more than 700 deaths were recorded in this initial outbreak. Scientists speculate that the SARS virus may have arisen in a sudden species jump from healthy civet cats to their owners in China in late 2002.

Symptoms of SARS are flu-like and include fever, chills, malaise, dry cough, shortness of breath, and headache. Liver damage and lymphopenia are also often present. Damage to the alveoli of the lungs is progressive and can be fatal. The virus spreads between humans by close contact, such as touching a contaminated surface or breathing in aerosol droplets created by the coughing or sneezing of an infected individual. At the cellular level, the SARS virus replicates chiefly in respiratory epithelial cells, although other tissues (such as eyes, heart, liver, kidney) and other cell types (such as macrophages) may also be targeted. Unlike most coronaviruses, the cytopathic SARS virus can be cultured *in vitro*, aiding the study of its infection mechanisms.

**iim) Smallpox virus.** The smallpox virus (variola) was introduced in Chapter 1. Although this virus has been eliminated globally in the wild, in this age of bioterrorism, it behooves us to briefly comment on it. Variola is a highly infectious member of the DNA poxvirus family. Smallpox disease is characterized by pain, fever, and a severe blistering of the skin that leaves disfiguring marks, particularly on the face of the infected individual (refer to Plate 1-1). Smallpox infection is fatal in 30% of cases. There is controversy over whether stocks of variola should be maintained for vaccine development. More on this issue appears in Chapter 23.

**iin) Vaccinia virus.** As recounted in Chapter 1, Jenner used the cowpox virus to vaccinate against smallpox back in the late 1700s. In modern times, the vaccinia virus is used. Vaccinia is an enveloped, double-stranded DNA virus and a member of the poxvirus family that includes cowpox and variola (smallpox). Curiously, it is not clear how the vaccinia

virus arose. Some researchers have speculated that early attempts at developing a better smallpox vaccine resulted in a recombination of cowpox virus with another poxvirus that resulted in the vaccinia virus. While vaccinia does replicate in human cells, it usually causes relatively mild illness (rash, low fever, enlarged lymph nodes, papule at the site of vaccination) and is only very occasionally fatal. As described in more detail in Chapter 23, vaccinia is under intense investigation as a vector for DNA vaccines.

## II. EFFECTOR MECHANISMS

It should be noted that not all of the effector mechanisms described in the following sections will apply to the resolution of all virus infections, depending on the type of virus and the particular host it is infecting at the time. However, contributions by CTLs and NK cells will likely play a role at some point in every infection, whether that contribution takes the form of cytolysis or cytokine secretion. Antibody defense, particularly neutralization, is crucial for protection against re-infection with many viruses. We also remind the reader that members of the TLR family of PRRs play a key role in both the innate and adaptive antiviral responses. TLR9 recognizes CpG-containing viral DNA, while TLR3 binds to double-stranded viral RNA, and TLR7 and TLR8 are triggered by single-stranded GU-rich viral RNA. TLR-stimulated macrophages and DCs produce large quantities of pro-inflammatory cytokines such as IFNα, IL-6, TNF, and IL-12. In the presence of these cytokines, TLR-stimulated DCs acquire viral components and are induced to mature, becoming competent APCs able to present viral antigens to naive T cells. The major antiviral effector mechanisms are summarized in Figure 22-5.

### i) IFNs and the Antiviral State

Interferon secretion is one of the earliest means by which the body defends itself against virus infections. As we learned in Chapter 17, the type I interferons were first discovered on the basis of their antiviral activities. Host cells infected with a virus produce IFNα and IFNβ, which in turn initiate a series of metabolic events in neighboring cells that result in their adopting an *antiviral state*. The reader will recall from Chapter 17 that IFNα and IFNβ induce the synthesis of the host enzyme PKR, which is inactive until it encounters a dsRNA molecule such as a viral genome. Among other substrates, activated PKR phosphorylates first itself and then eIF2, a factor necessary for translation of both host and viral proteins. Since the virus relies on the host cell's translation machinery for viral protein synthesis, viral reproduction is halted. IFNs also induce expression of 2-5A synthetase, an enzyme activated by dsRNA that generates short 2′,5′ oligoadenonucleotides. These 2-5A molecules activate RNAse L, which degrades both viral and cellular RNAs, again blocking translation. Surprisingly, mice deficient for PKR are capable of mounting effective immune responses to almost all viruses (except *vesicular stomatitis virus*, VSV), suggesting that redundancy for PKR function or a parallel antiviral pathway exists. These matters are under investigation.

As well as their involvement in the antiviral state, IFNs trigger the expression of multiple genes with profound effects on both the innate and adaptive immune responses (see Ch.17). These effects combine to control infection by a very broad spectrum of viruses. IFNs enhance phagocytosis, regulate the production of cytokines and NO, and promote the differentiation of monocytes into DCs. IFNγ is crucial for the mounting of a Th1 response necessary to support cell-mediated defense, consistent with the finding that mice lacking IFNγR are highly susceptible to vaccinia infection. IFNα has been used with success in the clinic against hepatitis infections, emphasizing the importance and utility of this family of molecules.

### ii) NK Cells

Because viruses are intracellular pathogens, cell-mediated mechanisms are of key importance in their elimination. While CTLs are the prime mediators of antiviral immunity, there is often a 4–6 day delay before these cells can expand to sufficient numbers to successfully eliminate the virus-infected cells. In situations in which virus infection causes the downregulation of MHC class I on the host cell surface, direct cytolysis of infected cells by NK cells (via ADCC or natural cytotoxicity) and NK production of inflammatory cytokines become of paramount importance. For example, human patients lacking NK cells show increased susceptibility to virus infections, especially herpesviruses.

### iii) $CD8^+$ T Cells

The intracellular nature of viral replication means that viral antigens are processed by the host cell's endogenous antigen-processing pathway and peptides are displayed on MHC class I on the surface of an infected cell. The viral peptides that constitute the T cell epitopes may be derived from almost any internal, structural, or envelope protein of the virus, although nucleoproteins and matrix antigens are favored. CTLs kill most virus-infected cells using perforin-mediated cytotoxicity or by inducing Fas-mediated apoptosis, but also secrete the cytotoxic molecules granulysin, TNF, and IFNγ. The objective is to rapidly kill an infected host cell prior to virus assembly and thus block viral spread. Studies of knockout mice lacking either CD8 or β2m (and thus MHC class I) have shown that these animals are highly susceptible to virus infections.

An interesting twist to CTL-mediated elimination of viruses arises with respect to hepatocytes. It seems that, unlike the case for any other cell type, low levels of viruses infecting hepatocytes are controlled solely by cytokine action that does not lead to cytolysis. For example, LCMV and mouse CMV are cleared from murine liver cells primarily by the action of CTL-synthesized IFNγ, in sharp contrast to the cytolytic elimination of these viruses in other cell types. In a poorly defined mechanism, the binding of IFNγ to its receptor on an infected hepatocyte activates intracellular degradation of viral components, curing the cell without killing it. In the case of hepatocytes infected with HepB virus, both TNF and IFNγ must

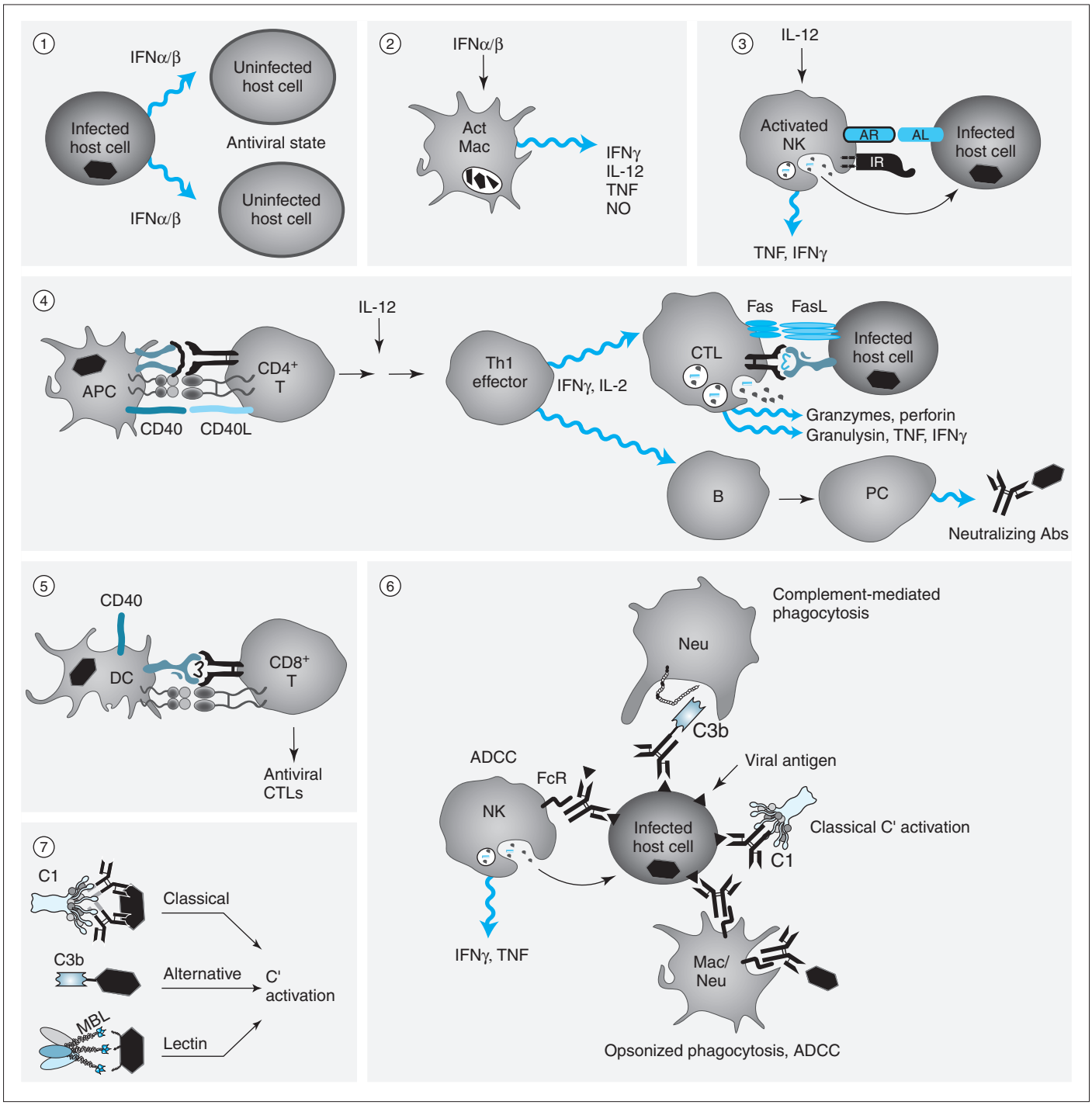



Figure 22-5

**Major Mechanisms of Immune Defense against Viruses**

(1) IFNα/β secreted by infected host cells induces an antiviral state in neighboring uninfected host cells. (2) IFNα/β also activates macrophage killing and production of pro-inflammatory cytokines and NO. (3) IL-12 produced by macrophages activates NK cells that secrete additional cytokines and use natural cytotoxicity to kill infected host cells that fail to express MHC class I. (4) Infected APCs present viral peptides to $CD4^+$ T cells that supply CD40L contacts required for licensing APCs. The $CD4^+$ T cells are activated by this encounter and generate Th1 effectors that supply cytokine help to Tc cells and B cells. Antiviral neutralizing antibodies block further spread of the virus, and antiviral CTLs secrete cytotoxic molecules and kill virus-infected host cells by Fas killing or perforin/granzyme-mediated cytotoxicity. (5) DCs infected by a "strong" virus upregulate their costimulatory molecules even in the absence of CD40 ligation, and activate Tc cells in absence of Th help. (6) Antibodies bound to a viral antigen on the surface of an infected host cell may engage the FcR on an NK cell, macrophage, or neutrophil and trigger ADCC or opsonized phagocytosis by these cells. Alternatively, the bound antibody may be bound by free C3b, facilitating opsonized phagocytosis of the infected cell. If the bound antibody binds C1, classical complement activation can lead to MAC-mediated destruction of the infected cell. (7) Complement may also be activated by the binding of C1, C3b, or MBL to the virus itself (as opposed to the infected host cell).

bind to their respective receptors on the hepatocyte surface to induce selective degradation of the viral genome as the virus replicates within the hepatocyte. Again, the threat is removed without killing the liver cell. Liver cells are killed when viral loads are high, because the heightened inflammatory response sparked by large numbers of virus particles recruits activated macrophages. In the cytokine-rich milieu of the inflamed liver, the macrophages commence expression of CD40L, which engages CD40 expressed on hepatocytes. This interaction induces FasL expression on hepatocytes, making them vulnerable to Fas-mediated apoptosis. Should FasL be engaged either by Fas on the surface of the same hepatocyte, or on an adjacent hepatocyte, cell death is induced, which can eventually lead to clinical liver damage. This type of liver pathology is prominent in severe cases of HepB and HepC infection in humans.

Immunopathology following CTL activation also arises with HTLV-1 infection. The vast majority of people infected with HTLV-1 go on to become carriers of the virus. In about 3% of carriers, the virus causes the adult T cell lymphoma/leukemia that gives this virus its name (see Ch.30). However, another 2.5% of carriers develop a chronic neurological disease called HAM/TSP (*HTLV-associated myelopathy/tropical spastic paraparesis*). The principal clinical feature of HAM/TSP is chronic inflammation of the spinal cord. It has been postulated that those individuals who mount a particularly strong CTL response restrict HTLV-1 spreading very efficiently, reducing the concentration of viral antigens that might otherwise induce inflammation. In an individual mounting a less effective CTL response, viral antigens are allowed to accumulate. A continuous cell-mediated response is stimulated that is accompanied by large amounts of pro-inflammatory cytokines that result in inflammation and damage within the CNS of the patient.

Some virus infections are combated by CTL responses that do not require $CD4^+$ T cell help in either cytokine or intercellular contact form. DCs play prominent roles in such viral infections. For example, influenza virus on its own can activate DCs such that they strongly upregulate B7 costimulatory molecules even in the absence of CD40 stimulation. In other words, the DCs become fully mature APCs able to activate $CD8^+$ T cells directly, without the need for help (CD40L engagement) from $CD4^+$ T cells. A similar scenario holds for LCMV infection in mice. While antibody and $CD4^+$ T cell responses do occur in response to LCMV infection, it is the $CD8^+$ CTL cell response that is critical for defense. Studies have shown that strong primary CTL responses can be mounted to LCMV in the absence of $CD4^+$ T cell help, reflecting the putative ability of this virus to access and directly activate APCs.

### iv) Humoral Defense

Because a virus is an intracellular pathogen, it is often out of reach of antibodies during the primary adaptive response. Nevertheless, naive B cells may recognize viral components displayed on the surface of an infected cell or may encounter progeny virions as they are released from an infected host cell. In the presence of the appropriate T cell help, neutralizing antibodies are produced and released into the circulation and memory B cells are generated. In a secondary response, the reinfection of the host can be rapidly blocked by the circulating neutralizing antibodies. These antibodies bar access of the virus to host cell receptors, and can initiate classical complement-mediated destruction of enveloped viruses. Virus-bound antibodies also act as opsonins and engage Fc receptors on phagocytes, facilitating internalization and phagosomal destruction of the invader. Similarly, antibodies that bind to viral components displayed on the surfaces of infected host cells can engage the Fc receptors of NK cells, neutrophils, and macrophages, inducing cellular and thus viral destruction by ADCC.

Antibodies directed against viral Ti antigens can be of great value in host defense. Because Ti responses involve only a B cell, they can be mounted more quickly than Td responses (which require a T cell and a B cell). Thus, B cells producing anti-Ti antibodies may act relatively early during an infection to minimize the spread of the virus until antibodies against viral Td antigens can be synthesized. Viruses such as VSV, LCMV, and Coxsackie virus have highly repetitive antigenic Ti structures on their surfaces that can activate virus-specific B cells without T cell help. Limited isotype switching may even occur in response to these antigens. For example, rotavirus infection in mice leads to mucosal production of virus-specific IgA even in the absence of $CD4^+$ T cells. It is thought that cytokines such as IL-4, IFNγ, and TGFβ secreted by γδ T cells, mast cells, macrophages, and/or NK cells may be sufficient to support isotype switching in response to viral Ti antigens.

### v) $CD4^+$ T Cells

As was true for intracellular bacteria, whole virions or their components may be taken into a professional APC by receptor-mediated endocytosis or phagocytosis. The viral proteins are then subjected to the exogenous pathway of antigen processing, and viral peptides displayed on MHC class II can activate $CD4^+$ T cells. Th cells are important for defense against most viruses because these cells both license APCs for naive $CD8^+$ Tc cell activation and supply the T help required for humoral responses to viral Td antigens. For example, neutralizing antibodies play a key role in the elimination of many rhabdoviruses, and the production of these antibodies is completely dependent on $CD4^+$ T cell help. Similarly, humoral and cell-mediated responses to adenovirus infections depend on $CD4^+$ T cell help in the form of CD40L contacts. Mice deficient for CD40L are highly susceptible to adenovirus since the mutant animals fail to produce adequate titers of antibodies and mount only minimal CTL responses. Finally, as one might expect, CD4 knockout mice readily succumb to a broad range of virus infections.

### vi) Complement

Surface components of virus particles can directly activate the lectin and alternative complement pathways, leading to the lysis of enveloped viruses. In addition, opsonization of viruses by complement components C3b and C3d promotes phagocytosis by neutrophils and macrophages that bear complement receptors. Simultaneous binding of a C3d-coated virus particle to the

CR2 B cell coreceptor (CD21) and the BCR of a B cell promotes B cell activation and production of virus-specific antibodies. As mentioned previously, such antibodies can neutralize virions and trigger classical complement-mediated destruction of either the virus or the infected host cell displaying viral antigens.

## III. EVASION STRATEGIES

Viruses are the most devious of pathogens when it comes to evading the host immune response. Different viruses employ different tactics, and it is not unusual for a single virus to make use of several different mechanisms (Table 22-10). Viruses are not generally fazed by the barrier thrown up by intact skin or mucosae. Many viruses have evolved the capacity to bind to host cell receptors that facilitate viral internalization into epithelial cells. In addition, a broad spectrum of viruses comes equipped with enzymes that break down whole epithelial layers, allowing them access to deeper tissues. Viruses may also be introduced directly into the blood via insect or animal bites.

Once infection has occurred, many viruses hide from the immune system. Others confront the immune response head on by interfering with host cellular pathways. Indeed, it is hard to find a cellular pathway that is not subject to some kind of impairment by a virus. For example, each step of the MHC class I and class II antigen processing pathways can be disrupted, as can complement activation, host apoptotic pathways, antibody responses and cytokine/cytokine receptor signaling. Such strategies require substantial amounts of gene expression. For example, over 25% of the adenovirus genome is devoted to genes encoding proteins that block the host immune response. Some examples of viral strategies are discussed in detail in the following sections.

### i) Latency

A virus that can adopt a latent state escapes the attention of the immune response, at least temporarily. Many viruses have the ability to persist and even multiply in cells in a defective form that renders them non-infectious for a period of time. The virus then waits until conditions in the host, such as drug-induced immunosuppression or immunodeficiency associated with disease or aging, provide better odds of survival and transmission to new hosts. In most cases, latency involves the inactivation of viral gene transcription needed for productive infection and the subsequent expression of new viral *latency-associated transcripts* (LATs). Reversal from latency back to productive infection requires some type of reactivation of the productive infection genes that can only occur when the immune response is weakened.

Different viruses achieve latency in different ways. The retroviruses, like HIV, become latent by integrating a cDNA copy of their RNA genome into the DNA of a host cell in such a way that there is limited transcription of viral genes (see Ch.25). The herpesviruses, which are large, double-stranded DNA viruses with genomes encoding numerous viral proteins, rely on other means of transcriptional control. For example, both VZV and HSV are herpesviruses that can establish latent infections in the neuronal ganglia. Neurons express little or no MHC class I and so are relatively safe havens to which these viruses can retreat. The viral genome persists in only 0.01% of the neuron population but does not integrate into the host DNA. Rather, the viral genome forms a complex with host nucleosomal proteins that blocks transcription of productive infection genes. A new set of LATs is then transcribed to maintain latency. Another important human viral pathogen that adopts latency is CMV. Latent CMV takes up residence in leukocytes and endothelial cells concentrated around the kidneys, heart, and liver. It is not yet clear whether the CMV

Table 22-10 **Evasion of the Immune System by Viruses**

| Immune System Element Thwarted | Viral Mechanism |
|---|---|
| Passive physical barriers | Breach epithelial layer via insects, animal bites, and viral hydrolases |
| Detection | Adopt latency |
| Antibodies | Undergo antigenic drift or shift<br>Express viral FcR that blocks ADCC or neutralization<br>Block B cell signaling |
| DCs | Block DC differentiation, maturation, or migration<br>Inhibit DC expression of co-stimulatory molecules<br>Upregulate surface FasL |
| $CD8^+$ T cells | Infect host cells with very low MHC class I expression<br>Interfere with MHC class I presentation<br>Express viral proteins that interfere with pMHC binding to CD8 and TCR<br>Force internalization of pMHC |
| NK cells | Express viral homologue of MHC class I<br>Increase synthesis of HLA-E<br>Increase synthesis of MHC class I |
| $CD4^+$ T cells | Avoid infection of professional APCs<br>Interfere with MHC class II presentation<br>Express viral peptides that interfere with pMHC binding to CD4 and TCR |
| Complement | Express viral homologues of host RCA proteins<br>Alter synthesis of host RCAs<br>Bud through host membrane and acquire coat of RCAs<br>Inhibit C9 polymerization |
| Antiviral state | Block secretion of IFN$\gamma$<br>Interfere with PKR/2-5A pathway |
| Apoptosis | Block receipt of apoptotic signal<br>Interfere with death receptor pathways<br>Express enzymes that neutralize the effects of free radicals<br>Manipulate the host cell cycle |
| Cytokines and chemokines | Express competitive inhibitors of cytokines and chemokines<br>Block cytokine/chemokine transcription<br>Downregulate host cell cytokine/chemokine receptor expression |

genome integrates into the host DNA during latency, or whether there is complete silencing of productive infection genes. There is some evidence for LAT-like transcripts in cells infected with latent CMV.

EBV and *Kaposi's sarcoma herpesvirus* (KSHV; formerly called *human herpesvirus-8*, HHV-8) are herpesviruses whose latency is associated with the development of host tumors. As mentioned previously, EBV infection is linked with B cell lymphomas and nasopharyngeal carcinomas, while KSHV appears to cause the Kaposi's sarcoma often found in AIDS patients. Unlike the oncogenic retroviruses, however, the genomes of these oncogenic DNA viruses do not integrate into the host DNA. Productive infection is halted via downregulation of productive infection genes and subsequent upregulation of LATs. For example, EBV latency correlates with the downregulation of the EBNA3 family of viral antigens. It is these proteins that normally supply immunodominant peptides for EBV-specific CTLs. The virus then upregulates expression of EBNA1 (*EBV nuclear antigen-1*), a protein essential for EBV latency. However, as is described in more detail later, the structure of the EBNA-1 protein blocks its presentation on MHC class I molecules, obscuring the virus from CTL recognition.

### ii) Antigenic Variation

A common way for a virus to hide from the host immune system is to change its antigenic "stripe" over successive generations, expressing antigenically new forms of viral proteins that may not be recognized by existing memory lymphocytes or antibodies in populations previously exposed to the virus. Such antigenic variation as a means of viral survival is most likely to be important in hosts that are long-lived (such as humans). Host longevity means that multiple re-infections of an individual can occur, improving the chance that the virus will remain in circulation. This factor is particularly important if the virus lacks the ability to become latent.

Rapid modification of viral antigens through random mutations is known as *antigenic drift*. Antigenic drift usually involves proteins expressed on the surface of the virion that would normally be the target of neutralizing antibodies. Extensive antigenic drift is a hallmark of influenza virus, which lacks proofreading enzymes and thus experiences high rates of mutation of its RNA genome during replication. Minor mutations to the hemagglutinin and neuraminidase proteins present on the surface of the influenza virion arise at a rate of about 1 in $10^6$ virus particles. These minor variants often replicate preferentially in the host, as they cannot be neutralized by existing antibodies raised against earlier strains. New strains of influenza arising due to continual antigenic drift spread fairly quickly through a population and are responsible for localized influenza outbreaks. A similar form of antigenic drift can be observed in human rhinoviruses as well as in foot-and-mouth disease virus. HIV undergoes very rapid antigenic drift (even within a given infected individual) due to the error-prone reverse transcriptase involved in the replication of its genome (see Ch.25).

Perhaps unique to the influenza virus is its ability to undergo *antigenic shift*, another type of variation that is less frequent but much more radical and dangerous. Antigenic shift can occur because the influenza virus genome exists as eight separate single-stranded RNA segments, each of which encodes a discrete protein involved in viral function. With such a genetic structure, two different influenza strains that simultaneously infect a single host cell can undergo a reassortment (sometimes inaccurately called "recombination") of their genomic segments (Fig. 22-6). Virus particles containing new combinations of parental RNA suddenly arise, dramatically changing the spectrum of protein epitopes presented to the immune system. Another factor favoring antigenic shift is the ability of many strains of influenza virus to infect an intermediate host (such as a pig or chicken) before being passed on to humans. These animal hosts are known as *animal reservoirs*. Reassortment of gene segments can also occur in an animal reservoir, again leading to new combinations of proteins expressed in the progeny viruses. An antigenically novel flu virus can then infect the human population, safe from antibodies and CTLs raised during previous exposure or vaccination.

Because influenza infections occur seasonally, the appearance of entirely new strains from one winter to the next seemed very sudden to early workers in the field, initiating the use of the term "shift." Antigenic shifts were responsible for the global or "pandemic" outbreaks of influenza that occurred in 1918, 1946, 1957 (Asian flu), and 1968 (Hong Kong flu). As an example of how severe the influenza virus can be, consider the pandemic of 1918. Even with the relatively slow nature of international travel in the early 20th century, this version of the influenza virus swept around the world and killed close to 20 million people within a few months.

### iii) Interference with MHC Class I-Mediated Antigen Presentation

Antigen processing pathways offer a wealth of opportunities for a virus to sabotage the initiation of an adaptive immune response (Fig. 22-7). Without MHC class I expression, a peptide cannot be presented to either $CD8^+$ Tc cells or CTLs, so that many viruses have targeted various steps of MHC class I protein synthesis and peptide display. Some viruses (such as CMV) avoid MHC class I presentation of viral antigens by infecting mesenchymal or epithelial cells that have very low MHC class I expression. As a result, almost no viral antigens are displayed to alert passing T cells. Other viruses attempt to circumvent MHC presentation by producing viral proteins that resist proteolysis, meaning that peptides capable of fitting in the MHC binding groove are not generated. For example, as mentioned previously, an important protein expressed by latent EBV is EBNA-1, a molecule important for the maintenance of latent EBV in the host cell. The EBNA-1 protein contains about 200 Gly-Ala repeats that resist proteolysis, blocking the presentation of peptides from this antigen and thus the activation of T cells directed against it. Cytomegalovirus expresses a kinase called pp65 that phosphorylates a viral transcription factor such that it cannot be processed by the infected cell's antigen processing machinery.

Still other viruses prevent peptide loading by blocking the normal synthesis of MHC class I. Adenovirus makes a small protein called E19 that has a lysine-containing "ER retention motif." E19 binds to MHC class I molecules and the ER retention motif traps the complex within the ER, abrogating

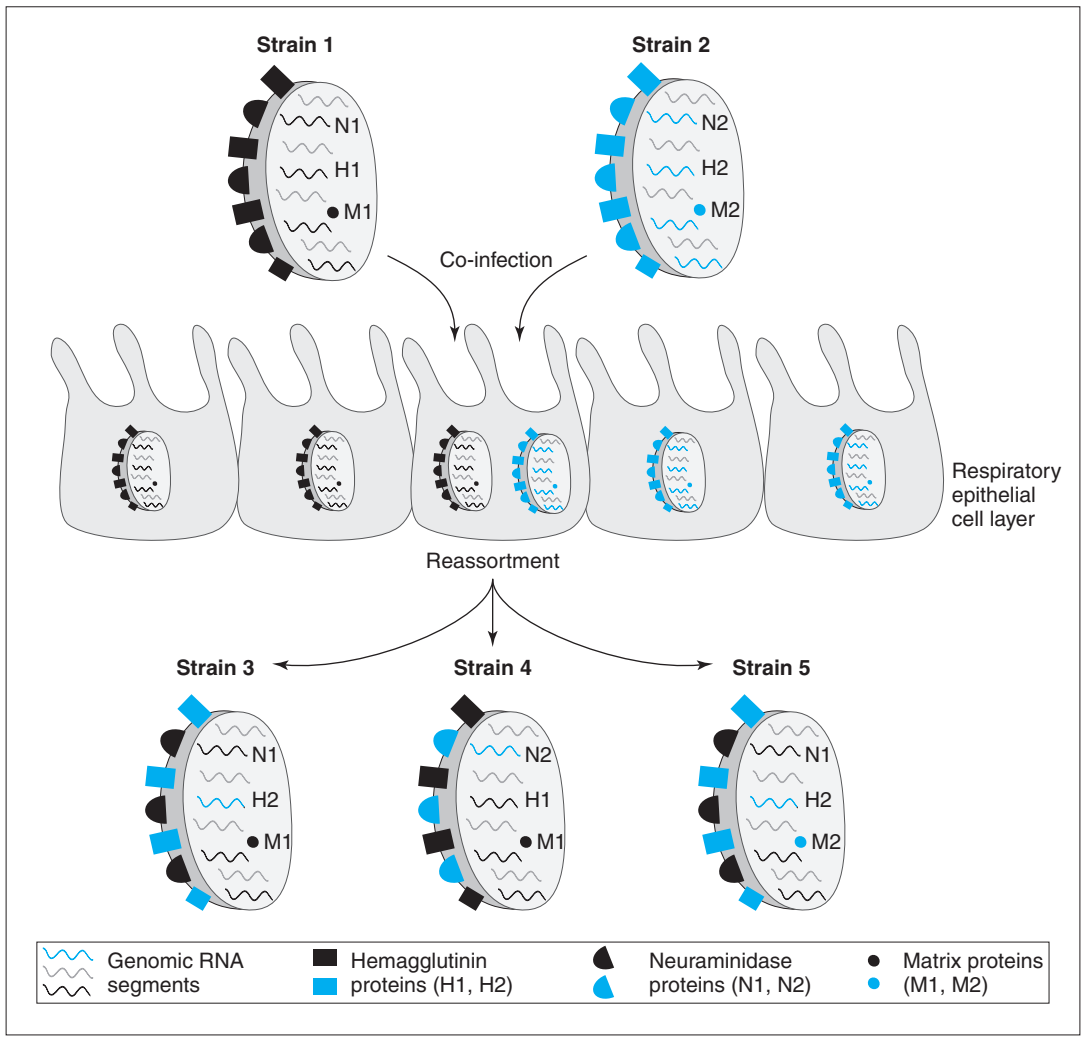



Figure 22-6

**Antigenic Shift**
The genome of the influenza virus is composed of eight segments that can reassort if two different viral strains infect the same cell. Progeny viruses acquiring various combinations of parental segments may express new constellations of proteins (not all possible combinations are shown here). Although internal genes like that encoding the matrix protein can also reassort, clinical immunologists define a particular antigenic shift by the identity of the hemagglutinin (H) and neuraminidase (N) molecules, since it is the presence or absence of B cell memory to these surface glycoproteins that influences the production of neutralizing antibodies.

peptide display on the cell surface so that CTL killing does not occur. In contrast, human CMV produces glycoproteins (such as the US3 protein) that induce the deglycosylation of some classes of newly synthesized MHC class I chains. The abnormal MHC class I proteins are then conveyed to the proteasomes for degradation. Similarly, the Vpu protein of HIV (see Ch.25) destabilizes newly synthesized MHC class I molecules, preventing them from ever reaching the surface. Some murine CMV proteins such as m06 bind tightly to MHC class I chains and act as lysosomal targeting sequences, causing the MHC class I chain to be destroyed in this organelle.

Another way to interfere with the MHC class I pathway is to alter the interaction of the peptide and the TAP transporter complex. As we learned in Chapter 11, peptides generated in the cytosol must be imported via the TAP machinery into the lumen of the ER for attachment to MHC class I and eventual presentation on the target cell surface. The process occurs in two steps: the ATP-independent binding of peptides to the TAP heterodimer and the ATP-dependent translocation of the peptides into the lumen of the ER. In EBV-infected B lymphoma cells, the transcription of both TAP-1 and TAP-2 is downregulated, reducing peptide loading and thus presentation of viral antigens to $CD8^+$ T cells. Herpesviruses express small proteins that interfere with peptide binding to TAP on the cytosolic side of the ER. Other viruses express proteins that allow the peptide to bind to TAP but then trap the complex on the luminal side of the ER, blocking its release and subsequent interaction with MHC class I.

MHC class I presentation can also be inhibited by events closer to the cell surface. For example, a mouse CMV protein called m04 associates with mature peptide–MHC class I structures at the cell surface, interfering with TCR- and/or coreceptor-mediated interactions with $CD8^+$ T cells. Another example is the multifunctional Nef protein of HIV (see Ch.25). Certain amino acid motifs in Nef promote its association in the plasma membrane with the host clathrin proteins involved in receptor-mediated endocytosis. Other sequences in Nef allow it to bind to certain MHC class I molecules (among other proteins; see later). This Nef-mediated physical connection between MHC class I and clathrin forces the internalization of the MHC class I molecule. The lysosomal degradation of MHC class I follows, so that the surface level of MHC class I is drastically decreased.

One last note: in addition to sabotaging MHC class I antigen presentation, HIV attempts to avoid CTL attack by

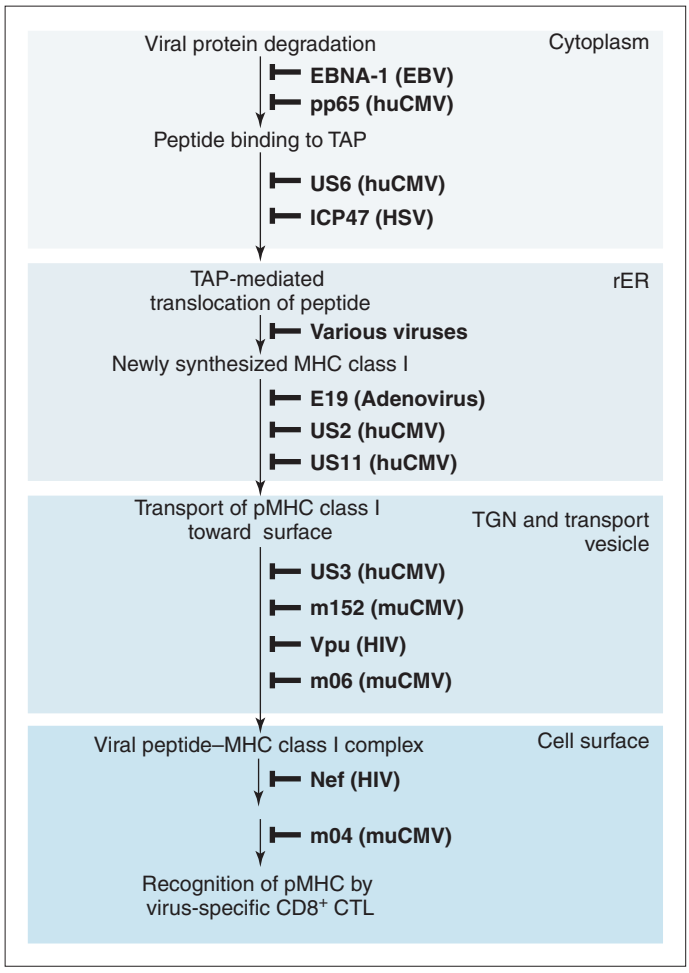



Figure 22-7

**Viral Interference with the Endogenous Antigen Presentation Pathway**
The boxes of increasingly darker shading represent the major compartments in which viral peptides are processed and loaded onto MHC class I for presentation on the infected cell's surface. Examples of proteins that inhibit events occurring in each compartment are indicated in bold, with the viruses from which they are derived shown in parentheses. TGN, trans-Golgi network.

producing variant peptides that act as TCR antagonists for HIV-specific CTLs (see Ch.25). Presentation of these peptides blocks the activation of the CTL subsets that recognize them, protecting the infected host cell from cytolysis.

### iv) Fooling NK Cells

Because NK cells are activated in the absence of MHC class I, they are crucial for the control of viruses that actively interfere with the processing of antigen and attachment to MHC class I. However, as one might expect, mechanisms exist that thwart this means of host defense, too. Human CMV expresses an MHC class I-like surface molecule called UL18 that engages the NK inhibitory receptor and fools the cell into thinking it has detected normal MHC class I (Fig. 22-8); the virus proceeds with its replication undisturbed. Poxviruses and herpesviruses also express MHC class I homologues, many of which have deficits in peptide binding. We mentioned previously that the Nef protein of

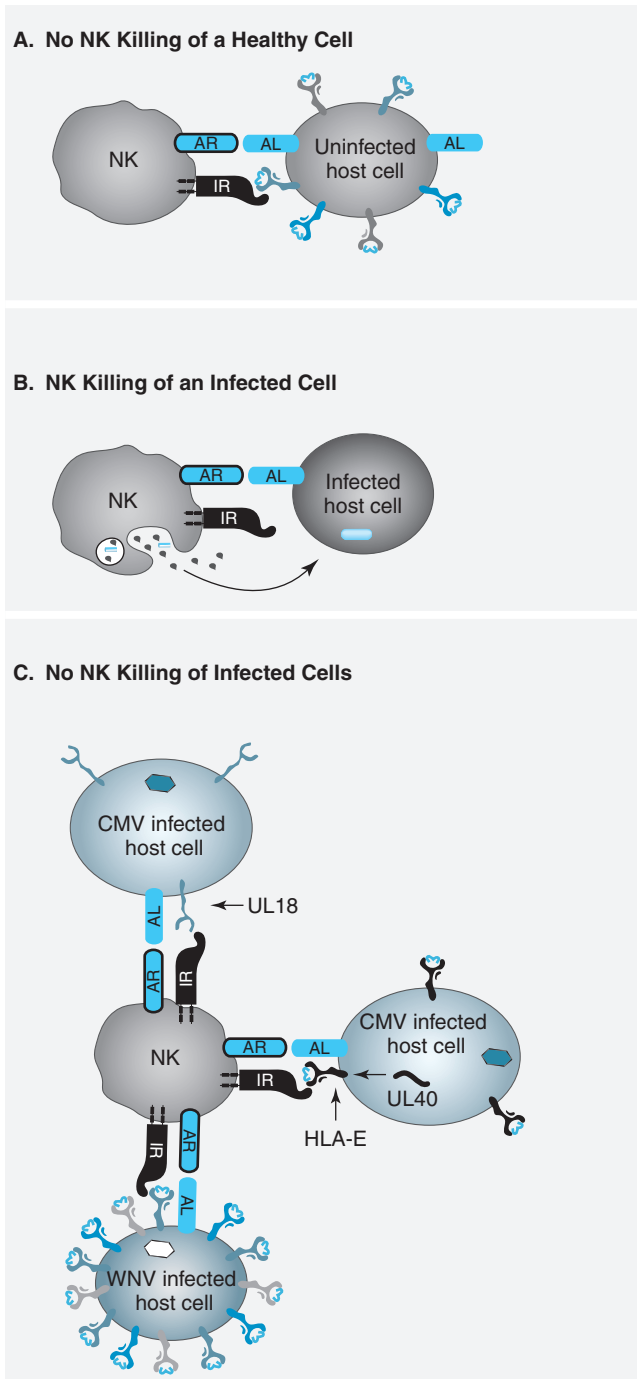



Figure 22-8

**Fooling NK Cells**
(A) An uninfected host cell expresses MHC class I molecules that bind to the inhibitory receptors (IR) of the NK cell and block signals mediated by the engagement of the activatory receptors (AR).
(B) Infected cells often lose expression of MHC class I, resulting in their destruction by NK cells. (C) Viruses have developed ways of engaging the IR and blocking NK killing. For example, human CMV induces both expression of the MHC class I analogue UL18 that can bind the IR, and the expression of UL40 that induces upregulation of HLA-E. In contrast, West Nile virus (WNV) induces overexpression of conventional MHC class I.

HIV depresses surface expression of MHC class I, blocking antigen presentation to CTLs. Interestingly, the expression of the non-classical MHC class I molecules HLA-C, HLA-E, and HLA-G is not affected by Nef, leaving at least some MHC class I-like molecules to bind to the inhibitory receptors of NK cells. The human CMV protein UL40 actually upregulates expression of HLA-E, increasing the numbers of non-classical MHC class I molecules able to bind NK inhibitory receptors. Curiously, some small viruses (such as the flavivirus West Nile virus) upregulate conventional host MHC class I expression in an infected cell in the hopes of staving off NK-mediated destruction. It is thought that this measure allows these rapidly reproducing viruses to complete their life cycle and disseminate long before the MHC class I-dependent CTL response can be brought to bear.

### v) Interference with MHC Class II-Mediated Antigen Presentation

Because $CD4^+$ Th cells support antiviral responses by delivering T help to antiviral B cells and CTLs, the MHC class II antigen presentation pathway has also become a target of viral evasion strategies. One of the easiest ways to block MHC class II presentation is to avoid getting antigens into professional APCs. For example, the rabies virus preferentially infects neurons and is very slow to replicate and lyse these cells, meaning that viral antigens are not easily collected by APCs until quite some time after the virus has entered the body. The natural immune response to rabies is thus considerably delayed. Interestingly, because the virus replicates relatively slowly, an individual bitten by a rabid animal can be successfully treated with a post-exposure vaccine, as an alternative to the administration of anti-rabies antibodies. The rabies vaccine contains an inactivated form of the virus that is injected directly into regions of the body where APCs are present, rapidly inducing an immune response (see Ch.23). Effectors activated by these APCs may then take steps to eliminate the rabies-infected neurons before the virus can spread further.

Other viruses take more indirect approaches to inhibiting MHC class II antigen presentation (Fig. 22-9). When a virus infects a cell, it produces IFNs that act on neighboring cells to induce the expression of many genes, among them MHC class II. As we learned in Chapters 10 and 17, IFNγ transduces its signals through the Jak-STAT pathway, activating the transcription factor CIITA required for MHC class II expression. The IE/E protein of human CMV is produced early during infection and blocks IFNγ signaling by destabilizing the Jak molecule, resulting in failed CIITA activation and thus reduced MHC class II expression. Proteins from adenovirus (e.g., E1A) can also affect MHC class II expression by influencing intracellular levels of activated Jak and STAT.

Although they differ in primary sequence, the secondary structures of newly synthesized MHC class I and II molecules are quite similar. This similarity allows the US2 molecule of human CMV to bind to MHC class II molecules and target them for proteosomal degradation before they can enter the endocytic compartment, as was true for MHC class I. In contrast, human herpes simplex virus-1 (HSV-1) expresses the gB viral protein that prevents MHC class II molecules from entering the endocytic system but does not trigger their degradation. Other viruses interfere with MHC class II presentation after the MHC molecule has entered the endocytic system. The pathway to peptide loading of MHC class II molecules is so complex that there are many opportunities for viral interference. The US3 protein of human CMV competes with invariant chain for binding to MHC class II. Papillomaviruses express small proteins such as E5 that disrupt the acidification of the endocytic compartments, inhibiting proteolysis and the generation of antigenic peptides. Similarly, the talented Nef protein of HIV can interfere with the proton pump required to acidify endosomes.

MHC class II molecules that have reached the cell surface are still not safe from some viruses. In addition to its ability to associate with the proton pump, HIV Nef induces internalization of surface MHC class II molecules just as it does for MHC class I molecules, forcing them back into the endocytic

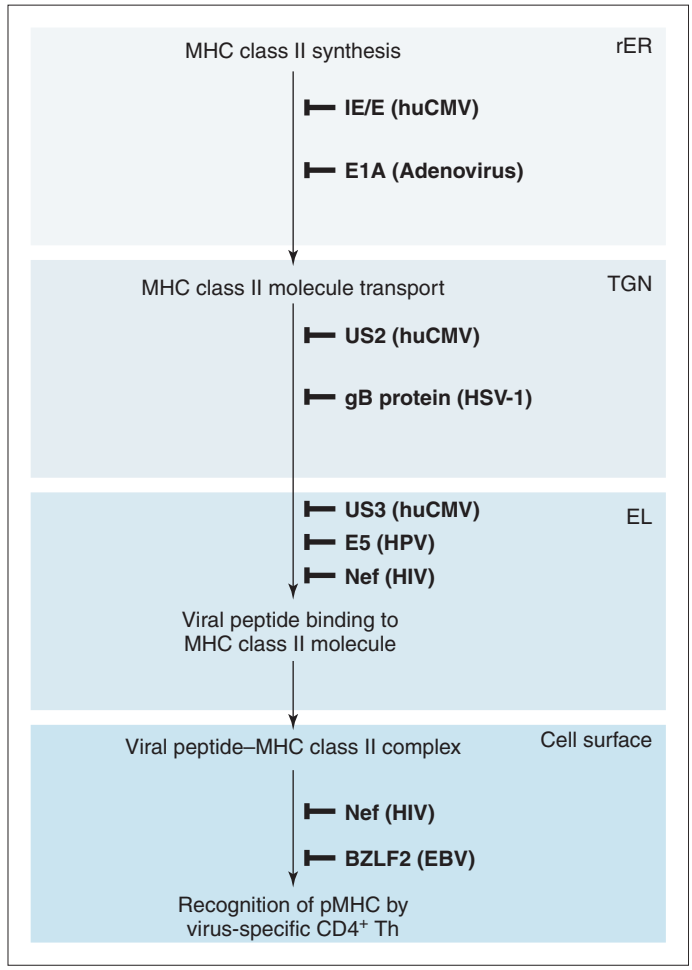



Figure 22-9

**Viral Interference with the Exogenous Antigen Presentation Pathway** The boxes of increasingly darker shading represent the major compartments in which viral peptides are processed and loaded onto MHC class II for presentation on the infected cell's surface. Examples of viral proteins that inhibit events occurring in each compartment are indicated in bold, with the viruses from which they are derived shown in parentheses. TGN, trans-Golgi network; EL, endolysosome.

system and to the lysosomes for destruction. EBV expresses a membrane glycoprotein called BZLF2 that interacts with MHC class II molecules intracellularly and on the cell surface. It is thought that this interaction inhibits antigen presentation to $CD4^+$ T cells, leading to a failure in T cell activation.

### vi) Interference with DCs

Some viruses block the initiation of immune responses directed against them by inhibiting the survival or maturation of DCs. For example, HTLV-1 infects DC precursors and prevents their further differentiation into immature DCs capable of capturing antigen. The infection of immature DCs by HSV-1 and vaccinia precludes DC maturation in response to cytokines. Other viruses, such as vaccinia and canarypox virus, kill DCs by inducing apoptosis. Measles virus forces DCs to form large aggregations called *syncytia* in which the virus replicates freely but further DC maturation is stymied. When human CMV infects a DC, it blocks its upregulation of costimulatory molecules so that the DC may anergize, rather than activate, any naive T cell it encounters. Alternatively, virus infection may turn the DC into a T cell killer. Infection by human CMV or measles virus upregulates the expression of FasL on the DC surface, forcing it to kill Fas-bearing T cells with which it comes into contact.

### vii) Avoiding Antibodies

Some viruses take the approach of interfering with B cell activation leading to antibody production. The nucleocapsid protein (NP) of the measles virus binds to the host FcγRIIB protein, which, as we learned in Chapter 9, exerts a negative regulatory effect on B cell activation. The binding of viral NP to FcγRIIB appears to enhance negative signaling such that B cell activation is dampened and antibody production is decreased. Other viruses (such as HSV-1) thwart antibody-mediated destruction by expressing viral Fcγ receptors on the infected host cell surface. These viral FcγRs are capable of binding to host antiviral IgG molecules that have bound viral antigen. Thus, when a host antibody binds to a viral antigen on an infected host cell surface in an effort to initiate complement-mediated lysis or ADCC, the viral FcγR binds to the Fc portion of the complexed IgG and makes it inaccessible. Neither ADCC nor classical complement activation can be triggered, and the infected host cell is not destroyed.

### viii) Avoiding Complement

Viruses use many of the same mechanisms as other pathogens to avoid complement-mediated destruction, and the reader is therefore referred once again to Box 22-3. Briefly, many viruses express homologues of the RCA proteins that control complement activation pathways, and some viruses directly inhibit host RCA proteins or alter the expression of these molecules. Other viruses fool the immune system by budding through the host cell membrane, acquiring the surface phenotype of the host and its membrane-integrated RCA proteins.

### ix) Counteracting the Antiviral State

Several viruses have developed mechanisms that disrupt the antiviral state. EBV blocks the initiation of the antiviral state by expressing the BARF1 gene. BARF1 encodes a soluble decoy receptor for the monocyte/macrophage growth factor CSF-1. BARF1 thus decreases the concentration of CSF-1 available to stimulate monocytes and macrophages. Macrophage proliferation and monocyte secretion of IFNα are inhibited, blocking the delivery of signals that protect neighboring cells. When HSV infects a cell that has already established the antiviral state (i.e., eIF2 has been phosphorylated by PKR), the virus expresses phosphatases that dephosphorylate eIF2, allowing viral protein translation to resume. The vaccinia and HepC viruses synthesize proteins that compete with PKR as sites for phosphorylation, while EBV and adenovirus express RNAs that bind to PKR but inhibit its activation. HSV also produces inhibitory analogues of the 2-5A molecules that block RNAse L activation, permitting viral protein synthesis to proceed.

Other viruses block host transcription, leading to establishment of the antiviral state. KSHV produces proteins that are homologous to the host IRF transcription factors. These viral proteins bind to IRF-binding sites on the host DNA but do not permit transcriptional activation. As a result, expression of IFN-inducible genes does not occur and the antiviral response is blocked. Similarly, adenovirus E1A proteins interfere with the formation of transcription factors required for the transcription of IFN-inducible genes, inhibiting host cell responses to the IFNs.

### x) Inactivation of CD4-Bearing Cells

Although HIV preferentially infects $CD4^+$ T cells, the very presence of CD4 appears to inhibit the release of newly assembled HIV virions from the T cell (see Ch.25). HIV thus has developed tactics for "going after" the CD4 molecule. The HIV Vpu protein promotes the degradation of newly synthesized CD4 molecules in the proteasome as it does for MHC class I, while the Nef protein promotes internalization and lysosomal destruction of surface CD4 as it does for surface MHC class I and II. Poxviruses and herpesviruses also produce proteins that trigger internalization and destruction of CD4. There is some debate as to whether the primary function of this mechanism is to promote the release of virions or to compromise T cell activation; both may be relevant.

### xi) Manipulation of Host Cell Apoptosis

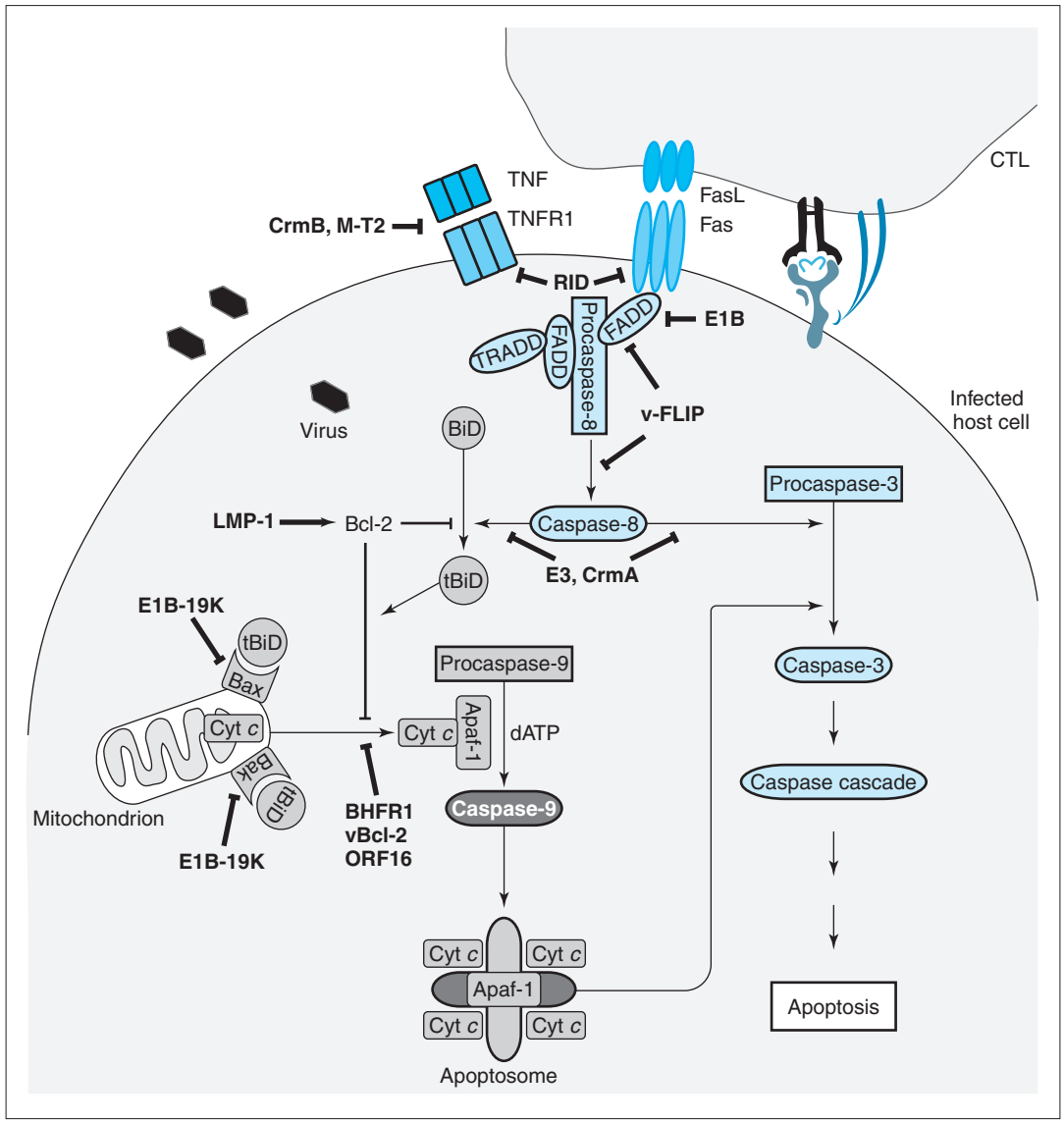
Host cell apoptosis prior to completion of replication is a distinct disadvantage for the more complex viruses with larger genomes, inspiring these viruses to develop means of blocking host cell death. Both genome size and tissue specificity of infection influence strategies to manipulate host apoptosis, since different apoptotic pathways may be triggered in different tissues by different stimuli. Host cell apoptosis is most commonly induced by CTL degranulation, NK cytolysis, Fas/FasL interaction, or the binding of TNF to TNFR. Many of the steps leading to apoptosis in each of these pathways are subject to viral interference (Fig. 22-10).

The extrinsic pathway of apoptosis induced by engagement of the death receptors Fas and TNFR is blocked by different viruses in different ways. Adenovirus expresses several E3 proteins that combine to form the *receptor internalization and degradation* (RID) complex. RID removes Fas and TNFR1 from the host cell surface by inducing the internalization of Fas and TNFR. These molecules are directed into the endosomes, followed by

Figure 22-10

**Viral Evasion of Host Cell Apoptosis**

A CTL often induces the death of an infected host cell via engagement of the death receptors Fas or TNFR1. Engagement of these receptors triggers the intrinsic and extrinsic pathways of caspase-mediated apoptosis. Examples of viral proteins that inhibit various steps of these pathways are shown in bold. See text for mechanisms and associated viruses.




transport to the lysosomes, where they are degraded. Cell death induced by an encounter with a T cell or monocyte expressing TNF or FasL is thus avoided. Several poxviruses express soluble or membrane-bound homologues of TNFR (such as CrmB; *cytokine response modifier B*) that act as decoy receptors for TNF and related cytokines. As was discussed in Chapter 17, myxoma virus produces a secreted homologue to TNFR called M-T2 that blocks TNF-mediated apoptosis.

Other viruses have targeted signal transducers further down the TNFR and Fas signaling paths. An adenovirus E1B protein blocks the oligomerization of FADD necessary for the activation of caspase-8. Several herpesviruses and poxviruses express proteins called vFLIPs (*viral FLICE inhibitory proteins*) that contain DEDs (*death effector domains*) capable of binding to FADD and caspase-8. Association with vFLIP blocks the initiation of the caspase cascade. Caspase-8 activation is also inhibited by the direct binding of the CrmA protein of cowpox virus, the E3 protein of adenovirus, and similar proteins in herpesviruses and vaccinia virus. These inhibitors are all *serpins*, members of the *serine protease inhibitor* superfamily.

We have mentioned in previous chapters the anti-apoptotic properties of the mammalian Bcl-2 protein. Bcl-2 blocks the intrinsic pathway of apoptosis by regulating the permeability of the mitochondrial membrane to ion traffic such that the release of cytochrome *c* from these organelles is inhibited. In the absence of cytochrome *c*, caspase-9 cannot be activated. Many viruses have sought to manipulate host cell apoptosis by either altering intracellular levels of host Bcl-2 or expressing Bcl-2 homologues. For example, herpesvirus samurai (HVS) expresses a homologue of Bcl-2 called ORF-16 that blocks mitochondria-mediated apoptosis. Two other examples can be found during EBV infection. In its productive infection phase, EBV expresses a Bcl-2 homologue called BHFR1 that blocks apoptosis induced by a variety of stimuli. However, during its latent phase, EBV expresses the LMP-1 protein instead. This molecule, which closely resembles a constitutively active CD40

molecule, is anti-apoptotic because it upregulates intracellular levels of host Bcl-2 and stimulates the activities of the host transcription factors NF-κB and AP-1; these transcription factors promote cell survival. This persistent stimulation by LMP-1 is thought to drive abnormal B cell survival and proliferation to the point of malignant transformation. A fourth example is the E1B-19K protein of adenovirus. E1B-19K binds to and inhibits those members of the Bcl-2 family that promote cell death, rescuing the host cell from apoptosis. Lastly, KSHV produces a protein called vBcl-2, which closely resembles human Bcl-2 and blocks apoptosis of infected host cells.

As well as by death receptor engagement, host cell apoptosis can be induced by external factors such as UV-irradiation (which generates free radicals) or the presence of chemically reactive molecules such as hydrogen peroxide. The genomes of several types of viruses contain genes whose products are homologous to cellular enzymes (such as glutathione peroxidase) and neutralize the effects of free radicals. Expression of such genes in transfected cells *in vitro* can protect them from cell death induced by UV-irradiation or hydrogen peroxide, but not apoptosis induced by TNF or Fas ligation. These anti-apoptotic mechanisms are likely to be relevant *in vivo* as well.

Sometimes a virus wants to induce the apoptosis of its infected host, especially a non-enveloped virus incapable of budding. Once the virus has completed replication and new virus particles have been assembled, the virus needs a way to get its progeny out of the cell. By inducing apoptosis of the infected cell, optimal dissemination of the progeny is achieved without the inflammation generated by necrotic death. An immune response is thus less likely to be mounted. Some examples of viruses that are thought to facilitate apoptosis in order to exit an infected cell include chicken anemia virus, Sindbis virus, HIV-1, and human parvovirus.

### xii) Manipulation of the Host Cell Cycle

Many viruses express proteins that interfere with regulation of the host cell cycle and subvert cell functions to viral use. Products of the tumor suppressor genes p53 and Rb (see Ch.26) are favorite targets. (p53 induces cell cycle arrest or apoptosis in response to DNA damage, and Rb blocks the division of cells with genetic abnormalities.) Interestingly, different proteins in the same virus can have seemingly opposing effects. For example, the E1A proteins of adenovirus influence the regulatory functions of p53 and induce p53-dependent apoptosis, while other adenovirus proteins bind to p53 and trigger its destruction. Adenovirus E1A proteins also bind to members of the Rb group and the p300/CBP family of transcriptional co-activators, disrupting host cell cycling. Similarly, the large T antigen of SV40 binds to both p53 and Rb proteins and inactivates them. In HepB virus, the pX protein binds to host p53 and either promotes or inhibits p53-mediated apoptosis, depending on the immediate microenvironment. In contrast, the E2 protein of HPV regulates p53-mediated cell cycle arrest, rather than apoptosis, in epithelial cells. The HPV E6 protein binds to p53 protein and targets it for ubiquitination and proteasomal degradation. In this case, levels of p53 are kept low enough in the infected cell that the cell can continue to divide, allowing further propagation of the virus.

### xiii) Interference with Host Cytokines

Early in viral infections, host cells are induced to produce copious quantities of cytokines with potent antiviral effects. Chemokines that govern the recruitment of infection-fighting leukocytes are also released. The inhibition of the production or action of cytokines and chemokines is thus a desirable strategy from the virus's point of view. For example, members of the poxvirus family evade immune surveillance by altering the local cytokine milieu and making it less favorable to the cellular cooperation that underpins an immune response. In particular, the IFNs, TNF, IL-1, IL-12, and various chemokines are targeted by different viruses. We have already mentioned that some viruses interfere with the pro-apoptotic signal transduction associated with TNF, and that both KSHV and adenovirus express proteins that inhibit IFN-inducible gene transcription. Poxviruses express a protein that blocks maturation of the precursor form of IL-1β, while a human CMV protein disrupts the transcription of chemokine genes. Other viruses have attempted to head off the host immune response by inducing the downregulation of cytokine receptor expression. For example, the surface expression of CXCR4 on $CD4^+$ T cells is decreased following infection by several types of human herpesviruses. Chemotaxis is inhibited and the calcium flux that supports intracellular signaling is suppressed.

Inhibition of IL-12 production is a major goal of many viruses since this cytokine is crucial for Th1 differentiation. For example, EBV synthesizes a homologue of the IL-12p40 subunit that can bind to host IL-12p35. The imposter may competitively inhibit the activity of host IL-12, contributing to a dampening of Th1 responses. EBV also produces a protein called BCRF1 which is homologous to mammalian IL-10. Like IL-10, BCRF1 suppresses IL-12 production by macrophages and IFNγ production by lymphocytes, further downregulating Th1 responses. The measles virus takes a different approach to IL-12 inhibition, at least *in vitro*. When the laboratory strain of measles virus binds to the RCA MCP (CD46) on cultured DCs and macrophages, IL-12 production by these cells is inhibited. Studies done *in vitro* to ascertain the underlying mechanism of this inhibition have shown that IL-12 synthesis can be impaired even in uninfected cells by the cross-linking of FcγRs, CD46, or the complement receptor CR3 on the host cell surface. It is speculated that the aggregation of CD46 by the measles virus delivers signals that block the transcription of both IL-12 subunits.

As discussed in Box 17-3 of Chapter 17, certain viruses appear to have appropriated and subverted mammalian genes for cytokines and cytokine receptors, producing proteins that are homologous to host cytokines and cytokine receptors. In general, the viral cytokines compete with host cytokines for host receptors, while the viral cytokine receptors divert the cytokine away from the host receptor. These interactions generally result in abnormal intracellular signaling and altered chemotaxis. For example, poxviruses synthesize a CC chemokine homologue called MC148 that binds with high specificity to CCR8 and blocks the chemotaxis of lymphocytes, macrophages, and neutrophils *in vitro*. On the other hand, HHV-6 synthesizes a viral cytokine U83 that binds to host CC or CX3C chemokine receptors but increases the calcium flux in an infected host cell. Chemotaxis of mononuclear cells to the site of viral infection

appears to be enhanced, providing the virus with a supply of new cells to infect. Sometimes a virus produces viral cytokines with seemingly opposite effects on chemotaxis. For example, KSHV produces a viral cytokine vMIP-II that antagonizes the CXCR5 and CCR5 receptors preferentially expressed on Th1 cells, suppressing their chemotaxis. However, KSHV also synthesizes another viral cytokine called vMIP-1 that binds to the CCR8 receptor expressed preferentially on Th2 cells, promoting the chemotaxis of these cells. Why KSHV should want to regulate Th cell behavior in this way is not known.

With respect to viral cytokine receptors, we have already mentioned the TNFR homologues produced by poxviruses and myxoma virus that block TNF from inducing host cell apoptosis. Similarly, vaccinia virus secretes an IFNα/βR homologue that intercepts IFNα, and an IFNγR homologue that binds IFNγ. Myxoma virus also produces huge amounts of an IFNγR-like viral cytokine receptor called M-T7 that binds host C, CC, and CXC chemokines, disrupting leukocyte extravasation into infected tissues. The p35 protein expressed by vaccinia and other poxviruses acts like a decoy chemokine receptor although it does not structurally resemble one. P35 binds to CC chemokines with higher affinity than does the host receptor, sequestering host chemokine molecules and reducing the efficiency of the immune response. The M-T1 protein of myxoma virus is another secreted molecule that binds and inhibits several chemokines *in vitro* and blocks the influx of monocytes into sites of acute infection *in vivo*. The opposite strategy has been adopted by KSHV. The suspected natural host cells of this virus are endothelial cells and B cells. KSHV induces the expression of a viral transmembrane receptor called ORF74 in the host membrane. ORF74 closely resembles CXCR2, an IL-8 receptor. However, unlike CXCR2, ORF74 is constitutively active, constantly stimulating signaling pathways associated with the synthesis of pro-inflammatory cytokines and *vascular endothelial growth factor* (VEGF). The increase in VEGF production is thought to trigger the formation of new sites of vascularization, facilitating the spread of the virus to fresh host cells. ORF74 may also regulate the migration of virus-laden B cells to lymphoid organs. Here, virions released from lysing host cells would find fresh, susceptible B cells to infect, enhancing propagation of the virus.

Other examples of virus-encoded homologues to mammalian immune system proteins may be found in the viral *semaphorins*. Mammalian semaphorins are signaling proteins that were first studied in neuronal cells but later identified in hematopoietic cells. The structurally diverse semaphorin superfamily includes secreted, GPI-linked, and transmembrane molecules, each of which contains a cysteine-rich "sema" domain of about 500 amino acids. The first recognized function of semaphorins was one of conveying signals guiding the growth of axons during embryonic neuronal development, but the fact that certain viruses have apparently acquired the genes for semaphorins implies the subversion of some kind of immunological function. The genome of vaccinia virus contains the gene for a small semaphorin called A39R that has a truncated sema domain. When A39R expression is engineered in human monocytes, this protein downregulates ICAM-1 expression, induces synthesis of IL-8, TNF, and IL-6, and inhibits migration in response to chemoattractants. The relevance of these activities to *in vivo* viral infections is under investigation.

## E. Immunity to Parasites

Parasites are some of the biggest killers in the pathogen pantheon and claim millions of lives every year, particularly in developing countries. An estimated 300–500 million people worldwide have contracted *malaria*, caused by the parasite *Plasmodium falciparum*. Over 1 million persons die of malaria each year, and many other infected individuals suffer greatly from severe lethargy that leaves them unable to work. Another 200 million people are infected with *Schistosomes* (blood flukes), and 800,000 die of these infections annually. *L. major*, which causes *leishmaniasis*, infects 2 million new hosts annually. The severity of the disease ranges from sub-clinical to mild to fatal even with treatment. Leishmaniasis was responsible for the deaths of 10% of the population of southern Sudan over 1995–2000. Even more worrying is the fact that this disease is on the rise, with a 500% increase in incidence reported in many endemic areas from 1993–2000. The development of clinical strategies to manage parasitic diseases occupies many medical researchers in the developing world.

### I. WHAT ARE PARASITES?

As mentioned at the start of this chapter, the term "parasite" covers a vast range of organisms that differ considerably in size, complexity, form, replication mode, and pathogenicity. At one end of the scale are the protozoans. Parasitic protozoan species are single-celled organisms whose behavior resembles that of bacteria or viruses. They often replicate directly within host cells, including within leukocytes. Important protozoan parasites for humans are *L. major*, *P. falciparum*, and *Trypanosoma brucei*, which causes *African sleeping sickness* (Table 22-11). At the other end of the scale, we have the parasitic worms. Many helminth species, which include the trematodes (flukes and worms that attack the lungs and liver), cestodes (tapeworms in the gut lumen), and nematodes (roundworms and hookworms, primarily in the intestinal lumen), are also major human pathogens. These organisms are complex, multicellular beasts that reproduce inside a host's body but outside its cells, or outside of the host entirely. However, in the latter case, millions of parasite eggs or larvae are often deposited in places (like water sources) where access to a host is made easy. The growth and maturation of the parasite then occur within the host.

Many parasites have multi-stage life cycles and operate through a *vector*, an intermediary organism that attacks the ultimate host. Indeed, each stage of a parasite may be able to infect a different host. This is a problem from a public health point of view, since a parasite that can make use of an invertebrate vector (such as a mosquito) or takes up residence in an animal reservoir (such as a raccoon) is much harder to deal with than a pathogen that infects humans only. As well as

Table 22-11 **Examples of Parasites and the Diseases They Cause**

| Parasite | Disease |
|---|---|
| **PROTOZOANS** | |
| *Entamoeba histolytica* | Amebiasis (enteric disease) |
| *Leishmania donovanii* | Leishmaniasis in viscera |
| *Leishmania major* | Leishmaniasis in face, ears, and skin |
| *Plasmodium falciparum* | Malaria |
| *Toxoplasma gondii* | Toxoplasmosis |
| *Trypanosoma brucei* | African sleeping sickness |
| *Trypanosoma cruzi* | Chagas' disease |
| **HELMINTH WORMS** | |
| *Ascaris* (roundworm) | Ascariasis (lung damage) |
| *Echinococcus* (tapeworm) | Alveolar echinococcosis (liver and lung damage) |
| *Onchocerca* (filarial worm) | African river blindness (eye damage) |
| *Schistosoma* (blood fluke) | Schistosomiasis (liver damage) |
| *Trichinella* (roundworm) | Trichinosis (intestinal damage) |
| *Wuchereria* (filarial worm) | Elephantiasis (lower trunk swelling due to lymphatic blockage) |

taking steps to fight pathogen infections of humans, measures to control the mosquito or raccoon population must be implemented. From an individual's point of view, that fact that some parasite stages may be intracellular while others are extracellular means that the infected individual must be able to mobilize both the cell-mediated and humoral arms of the immune response to eliminate the pathogen. The multiplicity of these factors makes it very difficult to design effective vaccines to combat parasites. We will now briefly describe some of the more important parasites and their modes of infection, before moving on to the immune responses required to control them.

The malarial parasite *P. falciparum* has a very complex life cycle (Fig. 22-11). When a female *Anopheles* mosquito bites an infected human to acquire a blood meal, it takes up RBCs bearing *P. falciparum* gametocytes. When the gametocytes reach the mid-gut of the mosquito, they commence maturation and 24 hours later combine to form worm-like zygotes that mature into *ookinetes*. Each ookinete penetrates into the mosquito's mid-gut wall, forms an oocyst, and starts to produce progeny called *sporozoites* within it. When an oocyst bursts, the sporozoites are released and migrate to the mosquito's salivary glands. When the infected mosquito bites a human, 5–20 sporozoites are injected into his or her subcutaneous tissues. Within minutes of injection, the sporozoites race through the blood to the liver and invade hepatocytes. In these cells, the sporozoites replicate asexually (and asymptomatically) for 2–10 days, producing thousands of *merozoite* progeny. The infected hepatocytes then lyse, each releasing 10,000–30,000 merozoites into the bloodstream where they invade erythrocytes and express parasite proteins on the RBC surface. Within the erythrocytes, the merozoites mature into *schizonts* that reproduce asexually and exponentially. The RBCs lyse 48 hours later, releasing new progeny merozoites into the blood, where they attack more erythrocytes. This synchronized rupture of the erythrocytes is felt as the cyclical constellation of chills, fever, and sweating that constitutes clinical malaria. These symptoms are due to the release of TNF and IL-1 by macrophages responding to the presence of lysed RBCs. However, not all RBCs are lysed by the merozoites. Within some infected RBCs, the merozoites develop into the sexual male and female gametocyte forms. These gametocytes can persist for years in the host. The cycle repeats when another mosquito bites the infected human and takes up RBCs infected with gametocytes.

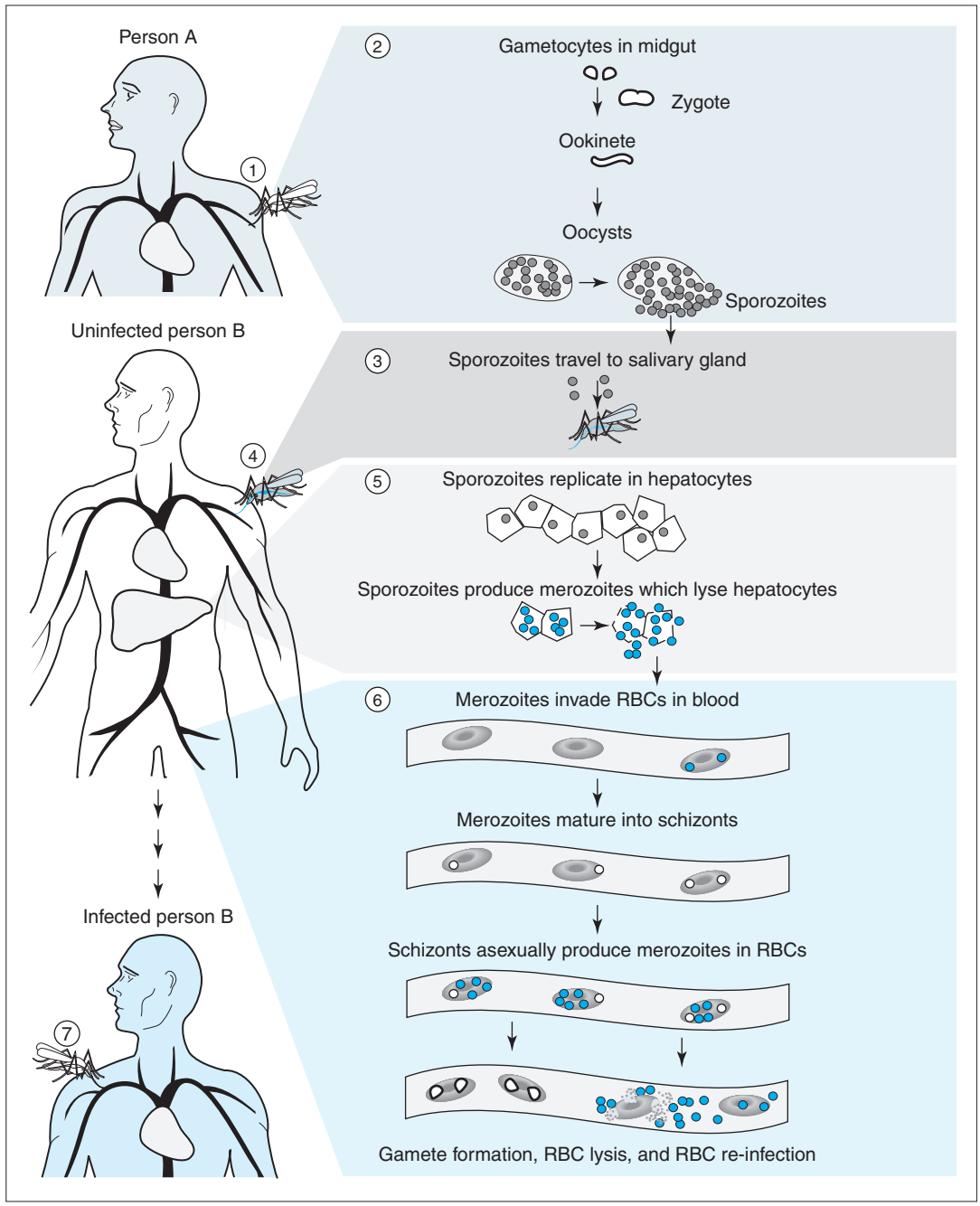
Interestingly, individuals that suffer from sickle cell anemia are resistant to malaria. "Sickle cell" refers to the abnormal shape of the erythrocytes in these individuals. This shape is caused by an altered hemoglobin protein that arises from a particular mutation in the hemoglobin gene. In addition to inducing sickling of the erythrocytes, the mutated hemoglobin protein appears to hinder the intracellular replication of the parasite. The sickle cell mutation occurs most frequently in Africa, where the advantage it confers over malaria has resulted in its selection despite its associated anemia. In other words, the prevalence of sickle cell disease in Africa is mainly the result of evolutionary pressure exerted by malaria. Other mutations in individuals naturally resistant to malaria inhibit the penetration of the erythrocytes by the parasite.

*Leishmania* are obligate intracellular protozoan parasites that preferentially invade macrophages in both humans and animals. These parasites have a two-stage life cycle and operate through sand flies as a vector. These insects introduce the parasite into the host when taking a blood meal. The *promastigote* stage is the form transmitted by the invertebrate vector, while the *amastigote* stage is the intracellular form that establishes infection in the vertebrate host. Depending on the species of *Leishmania*, the skin or the viscera of the host is affected. *L. major* causes cutaneous leishmaniasis, characterized by skin lesions and erosion of cartilage in the face and ears. Death can result from asphyxiation or starvation if much of the nasopharyngeal cartilage is destroyed. The less common visceral leishmaniasis is a systemic disease caused by *Leishmania donovani*. Splenomegaly, release of TNF, and severely decreased levels of leukocytes are its signature features.

*Toxoplasmosis* is caused by the spore-forming protozoan *Toxoplasma gondii*. This organism primarily infects and reproduces in cats. The parasite causes no illness in the infected cat, but when the parasite enters its sexual stage in the feline intestine, oocysts containing sporozoites are formed that are then shed into the feces. These feces may then contaminate food, water, or soil consumed or handled by other animals or humans. If a mouse drinks water contaminated with *T. gondii* oocysts and an uninfected cat eats that mouse, the cycle repeats in the new host. If an oocyst is ingested by a human, the sporozoites are released from the oocyst and travel through the blood and lymph to various tissues, causing infections of the eye, muscle, and brain. In healthy adult humans, the symptoms of

Figure 22-11

**Malarial Infection**
(1) Person A infected with *P. falciparum* is bitten by an uninfected mosquito that ingests RBC containing *P. falciparum* gametocytes. (2) Events in the mosquito gut. (3) Events in the mosquito salivary gland. (4) Infected mosquito bites uninfected Person B and injects sporozoites that travel to Person B's liver. (5) Events in person B's liver. (6) Events in person B's blood as he becomes infected. (7) Repeat of cycle when an uninfected mosquito bites infected person B.




*T. gondii* infection are usually mild and resemble mononucleosis at worst. However, in immunocompromised individuals (such as AIDS patients), sporozoites reaching the brain or eye can cause cerebral abscesses and neurological damage or retinitis leading to visual impairment. Pregnant women face the greatest danger from *T. gondii* infection (which is why mothers-to-be are warned not to change the litter boxes of their pet cats). Infection during pregnancy can cause severe brain damage or vision problems in the fetus or even stillbirth.

*T. brucei* is a flagellated protozoan parasite that uses the blood-sucking tsetse fly as a vector. Both humans and cattle can be bitten by the tsetse fly and thus be infected, causing devastation on two fronts in African villages. In the first stage of a human infection (which can extend for months or even years), the trypanosomes replicate freely in the blood and cause limited disease. Symptoms include fever, headaches, joint pain, and itchiness. However, in the second stage of the human disease, the parasite crosses the blood–brain barrier and attacks elements of the CNS. Victims show confusion, loss of appetite, lethargy, poor coordination, tissue wasting, and severe disruption of the sleep cycle (hence the name, African "sleeping sickness"). The disease is fatal without treatment, and irreversible neurological damage can occur if treatment is not started before the onset of the second stage. *T. brucei* can also cross the placenta and kill the developing fetus of an infected pregnant woman.

Another trypanosome, *Trypanosoma cruzi*, causes *Chagas' disease*. *T. cruzi*, which is carried by small blood-sucking insects called triatomines, is endemic in the southern United States, Central America, and South America. It is estimated that more than 20 million individuals are infected with *T. cruzi*, and that this parasite causes 50,000 deaths each year. The disease course has three stages: acute, indeterminate, and chronic. Only 1% of newly infected individuals show symptoms, including fatigue, fever, and a distinctive swelling at the site where the triatomine accessed the body. In most individuals, these difficulties are resolved without treatment. However, if *T. cruzi* is not completely cleared, the infection enters an indeterminate stage that can prevail for decades. The patient remains asymptomatic because the infection is kept under control by effective anti-parasite cell-mediated and humoral Th1 immune responses. About one-third of Chagas' disease patients go on to the chronic stage of the disease, in which much more serious symptoms appear 10–40 years after the initial infection. These patients exhibit a life-threatening inflammatory cardiomyopathy characterized by the infiltration of $CD8^+$ T cells and macrophages and the presence of DTH lesions. The esophagus and colon also become enlarged. Interestingly, *T. cruzi* itself is not found in high numbers in the affected organs, leading some researchers to suspect that the symptoms of chronic stage Chagas' disease may be autoimmune in origin. That is, high levels of parasites present at the acute stage of the infection prime the immune system to cause damage to self tissues many years later (see Ch.29). Indeed, healthy mice that receive isolated $CD4^+$ T cells from mice chronically infected with *T. cruzi* soon show myocardial pathology.

The *Schistosoma* worms (members of the trematode family) cause a disease called *schistosomiasis* in humans. The disease is associated with proximity to large, static bodies of water in developing countries because the intermediate host for these worms is a freshwater snail. Parasite eggs hatch in fresh water and penetrate into the snails. The larvae multiply in the snails, exit from the snails into the water, and attack and penetrate the skin of any nearby human. The immature schistosomes migrate first to the lungs and then to the liver, intestine, and blood. Eggs from reproducing schistosomes can get trapped in the walls of the intestine or the sinusoids of the liver, inducing a chronic inflammatory response. Products from the eggs can be directly toxic to liver cells, sometimes causing the host's death. To corral these egg products and block further development and dissemination of the parasite, granulomas form around the egg deposits. After several weeks, the granulomas are slowly replaced by collagen to form scars that can interfere with blood flow, particularly in the liver.

*Echinococcus* tapeworms, a genus of the cestodes, reproduce only in dogs but the eggs can be ingested by humans and cause alveolar *echinococcosis*. The eggs hatch in the human intestine and the larvae burrow through the intestinal wall into the lymphatic and blood circulatory systems. The larvae take up residence in various tissues and form large cysts, especially in the liver, kidney, lung, and brain. If a cyst ruptures, a sometimes-fatal IgE-mediated hypersensitivity reaction (see Ch.28) to the highly immunogenic contents of the cyst can occur.

A wide variety of helminth parasites is included in the nematode worm group, many of which cause well-known and severe diseases in humans. *Ascariasis* is an infection of the lung by members of the *Ascaris* (giant roundworm) genus. *Ascaris* eggs ingested by humans hatch into larvae that penetrate the intestinal wall, enter the blood, and travel to the lung. Severe inflammation in the lung can result from vigorous IgE-mediated attacks on *Ascaris* antigens in this location. *Trichinella spiralis* is another nematode whose larval form survives in cysts in uncooked meat. This parasite causes *trichinosis* when the meat is eaten and digested. The larvae develop into adults in the gut lumen, penetrate the intestinal mucosa, and produce more larvae in this location that enter the lymphatics and blood circulation. The larvae take up residence in muscles such as those of the tongue, diaphragm, and eye. *Onchocerca volvulus* is a nematode worm that causes *African river blindness*. Larvae are transmitted by African black fly bites to humans and the parasites migrate to the subcutaneous tissues. The larvae develop into adults that produce numerous microscopic thread-like progeny called *microfilariae*. The microfilariae can attack the eye, inducing inflammation that can rapidly lead to blindness. *Elephantiasis* is a severe swelling of the lower limbs and trunk that results from the blockage of the lower lymphatic vessels by the microfilariae of the worms *Wuchereria bancrofti* and *Brugia malayi* (Plate 22-5). These worms are transmitted by mosquitoes.

## II. EFFECTOR MECHANISMS

Different parasites evoke different types of immune responses. Some are countered by both humoral and cell-mediated responses, while others are best contained by one or the other. Obviously, the cellularity of the beast, the stage of life cycle, and whether that stage is intra- or extracellular will have a bearing on the defense mechanisms used at any one time. In general, protozoan parasites tend to induce Th1 responses, while helminth worm infections are handled by Th2 responses.

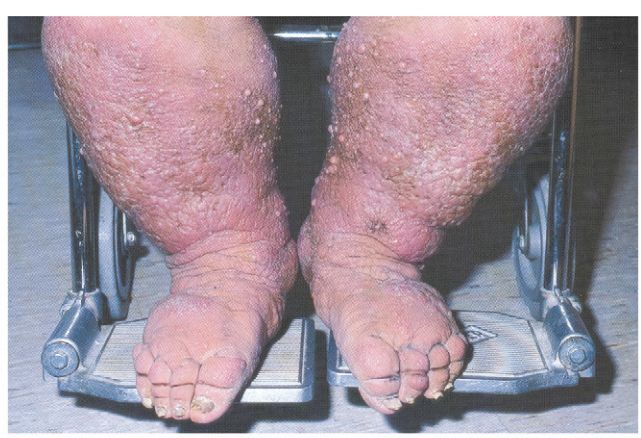


Plate 22-5

**Elephantiasis**
Reproduced with permission from Cooke R.A. and Stewart B. (2004) "Colour Atlas of Anatomical Pathology," 3rd edn. Elsevier Science, Amsterdam.

However, for unknown reasons, most anti-parasite immune responses are not 100% effective in ridding the body of parasites. The immune system then works to keep parasite numbers at a very low level such that the host does not experience disease. Should the immune system of the host later be compromised in any way, the residual parasites may be able to multiply freely, establishing a recurrence of symptoms.

### i) Defense against Protozoans

**ia) Humoral defense.** Although most protozoan parasites adopt intracellular habitats for at least some of their life cycle, humoral immune responses to these organisms do occur. All the effector mechanisms ascribed to antibodies for defense against extracellular bacteria (refer to Fig. 22-2) apply to defense against small extracellular parasites. Both natural and antigen-specific antibodies may act as opsonins for the extracellular stage of protozoans which, unlike the helminths, are small enough to be phagocytosed. As well, ADCC, classical activation of the complement cascade, and neutralization have all been described as effective means of eliminating these organisms. For example, high titers of neutralizing antibodies that recognize epitopes of a large surface molecule called MSP1 expressed on the *P. falciparum* merozoite surface can block the infection of new erythrocytes by merozoites released from a lysing RBC. Some of these antibodies appear to result from CD1-mediated presentation of part of the MSP1 protein to NKT cells, which may supply help to B cells in the form of secreted IL-4. Complement-mediated lysis of merozoite-infected RBCs via the classical pathway also occurs. In addition to antigen-specific antibodies, there are abundant natural antibodies present in human serum that recognize widely distributed carbohydrate antigens. Parasites bearing these molecules in their cell walls can trigger both the classical and lectin pathways of complement activation.

**ib) IFNγ, Th1 responses, and macrophage hyperactivation.** Like many intracellular bacteria, some parasites (e.g., *L. major*) resist destruction within an ordinary phagosome and actually enjoy life within a macrophage. These protozoans are resistant to or fail to induce the ordinary respiratory burst in activated macrophages. Only in a hyperactivated macrophage are there sufficient levels of RNI to efficiently kill such parasites. TNF produced by hyperactivated macrophages also plays an important but ill-defined role in anti-*L. major* responses, since administration of anti-TNF antibodies to cultures of IFNγ-treated macrophages blocks their ability to kill *L. major*. If even hyperactivated macrophages cannot clear the infection, a granuloma is formed to contain the threat.

We saw for intracellular bacteria that the hyperactivation of macrophages requires a Th1 response. How does the anti-parasite Th1 response arise? In the case of *L. major*, the primary target of infection is the macrophage. However, *L. major* also infects Langerhans cells in the epidermis, which become the prime initiators of the T cell-mediated immune response to this organism. In mouse models, *L. major* infection of DCs immediately induces these cells to produce copious quantities of IL-12. This IL-12 drives activated anti-parasite Th0 cells along the Th1 path, an event absolutely crucial for defense against *L. major* because these cells secrete the IFNγ required to hyperactivate macrophages. Mouse strains that are abnormally susceptible to *L. major* infection, such as the inbred strain BALB/c, are naturally incapable of inducing Th1 responses and mount ineffective Th2 responses instead. Th2 responses are unhelpful in this situation because Th2 cytokines such as TGFβ, IL-4, IL-10, and IL-13 inhibit IFNγ production and suppress the activation of iNOS. The same effect can be demonstrated in knockout mice lacking the genes for IFNγ itself, molecules regulating IFNγ production, or components of the IFNγ receptor.

In addition to macrophage hyperactivation, IFNγ has other powerful effects that make it the key molecule in anti-protozoan defense (Fig. 22-12). First of all, IFNγ is directly toxic to forms of many protozoan pathogens, including the sporozoite form of *P. falciparum*. Studies *in vitro* have shown that IFNγ eliminates sporozoites from infected hepatocytes in culture, and mice treated with IFNγ prior to infection with sporozoites are partially protected. Secondly, IFNγ establishes a positive feedback loop of Th1 differentiation because it stimulates IL-12 production by DCs and macrophages. This IL-12 in turn triggers additional IFNγ production by NK and NKT cells. In fact, mice and monkeys treated with IL-12 alone are able to resist sporozoite infections. Thirdly, IFNγ is a potent inducer of iNOS in infected macrophages. Induction of iNOS results in the production of intracellular NO, which eliminates either the parasite itself or the entire infected cell. As well, IFNγ upregulates the expression of p47GTPases localized in the ER or Golgi. The p47GTPase Lrg47 is essential for defense against *L. major*, *T. gondii*, and *T. cruzi*. Lastly, IFNγ upregulates the expression of Fas on the infected macrophage surface, rendering it susceptible to Fas-mediated apoptosis when it contacts a FasL-expressing T cell.

The CD40-CD40L intercellular contact is also crucial for anti-parasite Th1 responses, as evidenced by the increased susceptibility of $CD40L^{-/-}$ and $CD40^{-/-}$ mice to *L. major* infection. Engagement of CD40 on macrophages and DCs by CD40L on activated T cells promotes the production of IL-12 by these APCs. In the absence of such engagement and IL-12 production, the Th1 response and IFNγ production cannot be sustained, and the animals become vulnerable to the parasite. Injection of IL-12 into CD40L-deficient mice can partially protect them against *L. major* infection.

**ic) $CD8^+$ T cells and γδ T cells.** In infected cells in which protozoan parasites escape from the macrophage phagosome into the cytosol, parasite antigens may enter the endogenous antigen processing system and be presented on MHC class I. $CD8^+$ T cells are then activated and respond to the threat. Indeed, acute disease in experimental mice caused by *L. major* or *T. gondii* is exacerbated if the host is depleted of $CD8^+$ T cells by injection of anti-CD8 antibodies prior to infection. However, CTL-mediated cytolysis is not actually very effective against acute protozoan infections. Rather, it is the secretion of IFNγ by activated parasite-specific CTLs that is important. Similarly, IFNγ secretion by γδ T cells has been implicated at the sporozoite stage of malarial infections, since parasite loads are increased in animals lacking these cells. It should be noted that, while perforin-mediated cytotoxicity is not effective in the acute stage of *T. gondii* infection in mice, this mechanism is important for controlling the chronic stages of infection.

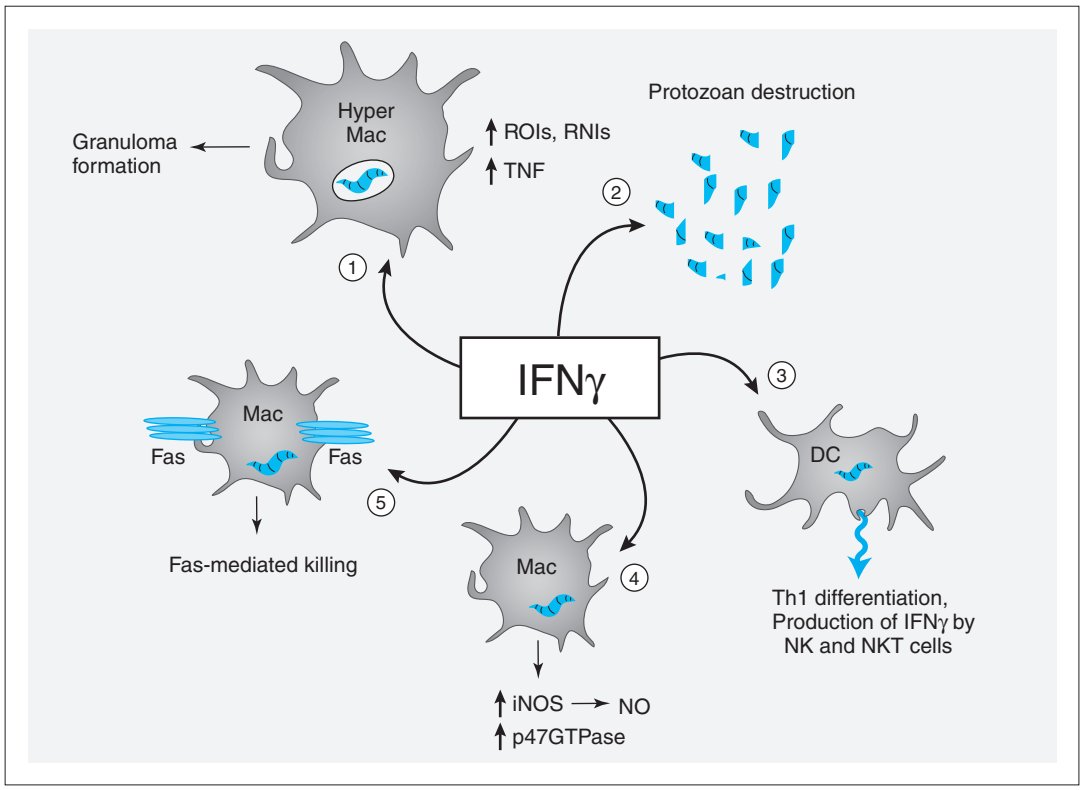



**Figure 22-12**

**Role of IFNγ in Immune Defense against Protozoan Parasites**

IFNγ produced by activated Th1 cells, CTLs, NK, and NKT cells is important for (1) hyperactivation of macrophages and granuloma formation that control resistant protozoans; (2) direct toxicity to many protozoan species; (3) stimulation of IL-12 production by infected DCs to sustain a feedback loop of Th1 differentiation; (4) induction of iNOS and increased NO production as well as increased p47GTPase activity in infected macrophages; and (5) induction of Fas expression on infected macrophages.

### ii) Defense against Helminth Worms

**iia) Unique Th2 responses.** While Th1 responses are best for combating protozoan parasites, Th2 responses are vital for defense against large, multicellular helminth worms. In fact, *in vitro*, antigens from the surface of a worm egg can trigger an abrupt switch from an incipient Th1 response to a Th2 response. Humans naturally resistant to *Schistosoma mansonii* exhibit high levels of Th2 cytokines, whereas those susceptible to this pathogen have high levels of Th1 cytokines. Furthermore, susceptibility to *S. mansonii* infection in humans has been found to be under the control of a gene called SM1. Interestingly, this gene occurs in a chromosomal region (5q31-33) that encodes several molecules that regulate T cell differentiation. It is speculated that SM1 may be involved in influencing Th1/Th2 immune deviation.

The anti-helminth Th2 response requires the involvement of IgE, eosinophils, and mast cells, a combination that does not contribute significantly to defense against other types of pathogens (Fig. 22-13). Activated $CD4^+$ T cells are critical for anti-helminth defense because these cells differentiate into effectors supplying the Th2 cytokines and CD40L contacts required for isotype switching to IgE by B cells. Circulating IgE directed against worm surface molecules can bind to the pathogen, attracting the attention of circulating eosinophils expressing FcεRI molecules at a moderate density. Alternatively, prior to encountering specific antigen, the anti-parasite IgE may bind to mast cells expressing high levels of FcεRI, allowing these cells to "pre-arm" against the parasite. In either case, the interaction of parasite antigen, anti-parasite IgE, and FcεRI triggers intracellular signaling within eosinophils and mast cells that induces degranulation in close proximity to the worm. Eosinophil granules contain a variety of chemical substances that work directly and indirectly to kill the worm. Some molecules degrade the skin of the worm, creating an opening that allows other eosinophils to penetrate into its underlying tissues. These cells also degranulate and release additional toxic proteins and peptides that kill the worm. In addition, certain enzymes released from the eosinophil granule can further stimulate the release of histamine by activated mast cells. Histamine causes the contraction of host intestinal and bronchial smooth muscles that is associated with peristalsis and bronchospasm. The actions of these muscles can shake the parasite loose from its grip on the mucosae and expel it from the body. Histamine is also directly toxic to some helminth parasites.

**iib) Roles of Th2 cytokines in anti-helminth defense.** The Th2 cytokines IL-5, IL-4, and IL-13 are vitally important for defense against helminth worms. IL-5 strongly promotes the proliferation, differentiation, and activation of eosinophils, and supports the differentiation of plasma cells that have undergone isotype switching to IgA production. As we learned in Chapter 20, IgA is the precursor to secretory IgA that coats the mucosae and fends off parasite attachment, particularly that of schistosome worms. In humans, IL-5 expression is strongly associated with resistance to *S. mansonii* infection. IL-4 and IL-13 suppress macrophage production of IL-12, inhibiting IFNγ production and hence the development of a Th1 response. IL-13 is also required for the development

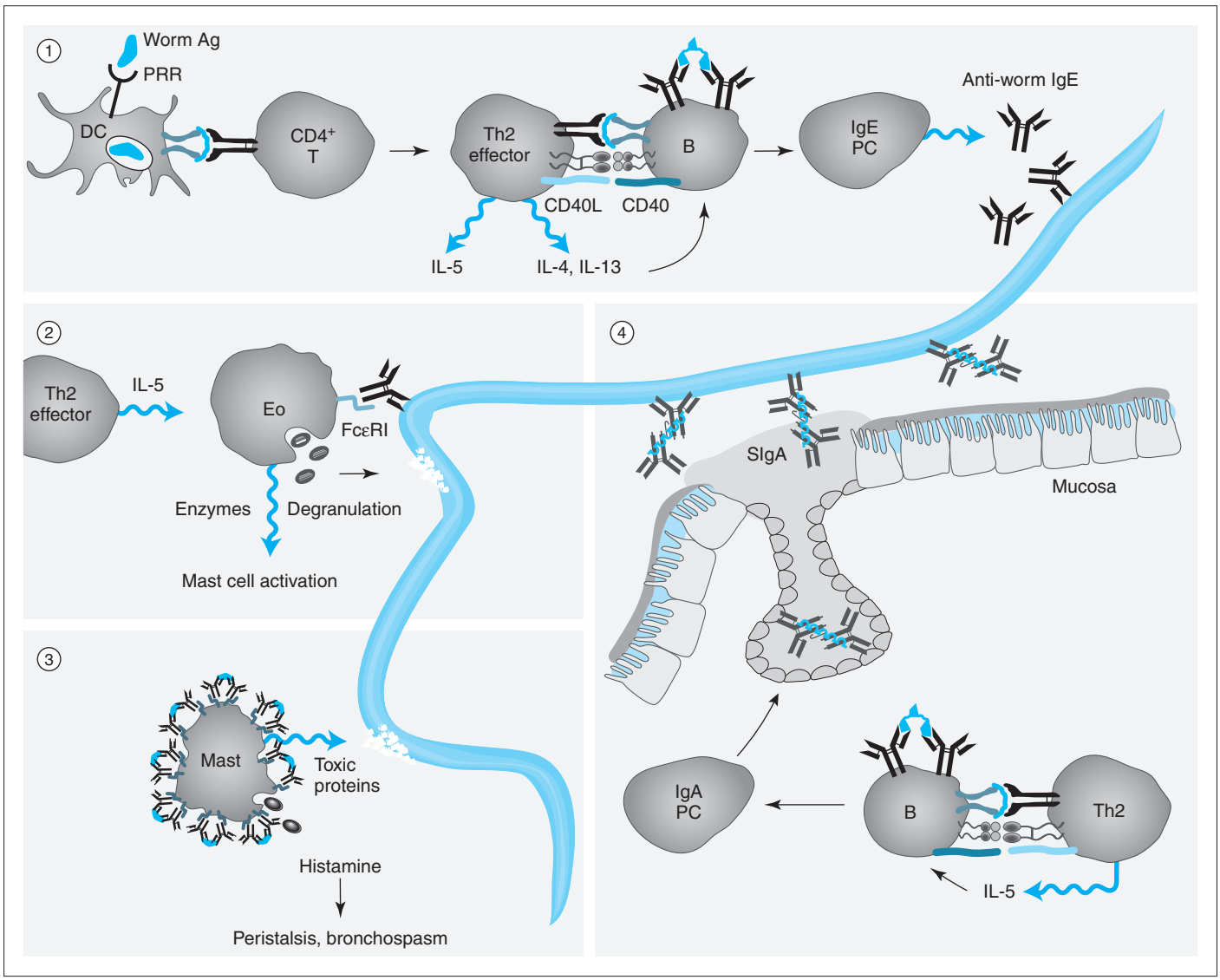



Figure 22-13

**Major Mechanisms of Immune Defense against Helminth Worm Parasites**
(1) DCs that have captured a worm antigen activate $CD4^+$ T cells that are induced to undergo Th2 differentiation. Th2 effectors produce cytokines that induce isotype switching in activated B cells to IgE. (2) IL-5 produced by Th2 cells activates eosinophils that bind to worm-bound antibodies via their FcεRIs. Eosinophil cytotoxic granules contain molecules that directly damage the worm surface and stimulate mast cells to degranulate. (3) Mast cells pre-armed with anti-parasite IgE are activated by worm antigens and release histamine, which induces gut and airway spasms to eject the worm. Mast cells also release proteins that are directly toxic to the worm. (4) IL-5 produced by Th2 cells also induces isotype switching to IgA in mucosal anti-worm B cells. Secretory IgA (SIgA) blocks the worm from gaining a foothold on the mucosal surface.

of airway hyperresponsiveness and the alterations to gastrointestinal fluids and the functions of intestinal smooth muscle and epithelial cells that accompany GI pathogen expulsion. Both IL-4 and IL-13 are effective in inducing Th2 responses to nematode and schistosome infections, but the response to a specific parasite may rely more heavily on one cytokine than the other. For example, IL-13 is more important than IL-4 for the expulsion of *Nippostrongylus brasiliensis* from the gut of mice, but either will do for the elimination of *Trichurus muris*.

## III. EVASION STRATEGIES

The fact that many parasites have multi-stage life cycles means that each pathogen has many different opportunities to thwart the immune response. Some important mechanisms underlying this evasion are summarized in Table 22-12. Because each stage of the parasite may employ multiple mechanisms and each parasite may have multiple life stages, natural immunity that successfully blocks parasite infection is difficult to establish in humans, and may take many years to develop even with ongoing exposure to the parasite.

Table 22-12 **Evasion of the Immune System by Parasites**

| Immune System Element Thwarted | Parasite Mechanism |
|---|---|
| Specificity of T and B cells | Have a multi-stage, multi-host life cycle<br>Lyse infected lymphocytes |
| Antibodies | Acquire host surface proteins that block binding of anti-parasite antibodies<br>Shed parasite membranes bearing immune complexes<br>Hide in macrophages<br>Modify surface proteins to cause antigenic variation |
| Phagolysosomal destruction | Block fusion of phagosome to lysosome<br>Escape from phagosome into cytoplasm<br>Resist lysosomal enzymes<br>Decrease respiratory burst<br>Lyse resting granulocytes and macrophages |
| Complement | Modify cell membrane so alternative pathway cannot be activated<br>Express RCA-like proteins<br>Degrade attached complement components and release the terminal complex<br>Force continuous complement activation so that components are exhausted |
| T cells | Induce accelerated apoptosis of memory T cells<br>Secrete immunosuppressive peptides |
| APCs | Interfere with DC maturation and macrophage activation<br>Promote IL-10 secretion by T cells to downregulate MHC class II<br>Decrease IL-12 production to reduce Th1 differentiation |

### i) Avoiding Detection

The simplest evasion strategy is, of course, to avoid detection in the first place. *L. major* hides from the immune response by sequestering itself within host macrophages. Some schistosomes are masked from immune surveillance by the surface acquisition of host self antigens such as blood group glycolipids and forms of MHC class I and II molecules. The dense "forest" created by these host molecules blocks the host antibodies from binding to parasite surface antigens. Other schistosomes (and other protozoans) repel antibody attack by shedding their membranes, ejecting the immune complex of the parasite antigen and host antibody.

Like viruses, parasites use antigenic variation to avoid detection. For example, *T. brucei* spontaneously modifies its expression of surface molecules to forestall recognition by circulating antibody. There are multiple (perhaps as many as 1000) separate genes encoding versions of the dominant surface glycoprotein VSG (*variable surface glycoprotein*) covering the trypanosome. Each trypanosome expresses only one copy of the VSG gene at a time, but regularly shuts down that gene and switches to another that results in a glycoprotein coat of a slightly different amino acid sequence. Antibodies raised to the first VSG protein may not recognize the second, allowing the parasite to escape recognition and thus destruction.

*P. falciparum* has evolved multiple means of antigenic variation to confound immune surveillance. First of all, different strains of *P. falciparum* express variant-specific forms of a large number of non-cross-reacting antigens on the surfaces of infected host erythrocytes. These strains tend to be endemic to a particular geographic region. An individual resistant to one strain of the parasite who travels to a new region will therefore likely be susceptible to the strain dominant in that region. Moreover, within the same host, successive populations of RBCs infected with the same strain of *P. falciparum* can express slightly different versions of certain parasite proteins. The expression of the novel antigens confounds antibodies previously raised against the antigens expressed in the first wave. In addition, many proteins necessary for parasite development are expressed only during the developmental stage in which they are required. This strategy minimizes the "window" of time during which a particular anti-parasite antibody can be effective. By the time antibodies to an early stage antigen are raised, the parasite may have either become intracellular or moved on in its life cycle such that the early antigen is no longer expressed.

### ii) Avoiding Phagolysosomal Destruction

Most parasites have developed strategies to avoid destruction by the effector cells in which they have hidden. The macrophage phagosomes in which *T. gondii* is contained are unable to fuse with lysosomes, sparing this organism attack by hydrolytic enzymes. Like many intracellular bacterial species, the protozoan *T. cruzi* enzymatically lyses the phagosomal membrane prior to lysosomal fusion and escapes to the cytoplasm of the host cell. While a significant proportion of early stage *L. major* promastigotes succumb to lysosomal destruction of phagosomal contents, the later stage is somehow able to resist digestion. There is evidence that the receptor used by the later stage parasite to access macrophages may not trigger a complete respiratory burst, making the phagosome a less hostile environment for the late stage invader. Some parasites, such as the intestinal protozoan *Entamoeba histolytica*, turn the tables and lyse resting granulocytes, macrophages, and lymphocytes with which they come in contact.

### iii) Avoiding Complement

Parasites have developed unique approaches to avoiding complement-mediated destruction. While the stages of many parasites that live in invertebrate vectors can be easily eliminated by MAC attack, those stages that infect vertebrate cells have modified their cell membranes so that these organisms no longer trigger the alternative complement activation pathway. Furthermore, there is evidence that the vertebrate stages of several parasites express a molecule that functionally resembles the mammalian RCA protein DAF. Still other parasites can proteolytically repel complement-activating molecules that have attached to their surfaces, or cleave the Fc portions of membrane-bound antibodies. For example, *L. major* can induce the release of the entire complement terminal complex from its surface. Some parasites can secrete molecules that force fluid phase complement activation, resulting in exhaustion of complement components.

### iv) Manipulation of the Host Immune Response

Active manipulation of the host immune response is a tactic used by some sophisticated parasites, including *P. falciparum*. Certain epitope variants produced by this parasite promote secretion by T cells of the immunosuppressive cytokine IL-10 rather than IFNγ, resulting in downregulation of MHC class II expression, inhibition of NO production, and decreased expression of pro-inflammatory cytokines by T cells. In addition, *Plasmodium*-infected RBCs express a parasite-encoded adhesion protein that allows the RBCs to bind to integrins on the surfaces of DCs and macrophages. This binding blocks the activation of macrophages and interferes with the maturation of DCs, thus inhibiting APC function and T cell responses. The erythrocytic stage of malaria infection can also induce the accelerated apoptosis of antigen-specific memory T cells. *L. major* uses slightly different tactics to manipulate the host immune response. The reader will recall from our previous discussion of viral evasion strategies that the cross-linking of CD46, complement receptors, or FcγRs on macrophages by viral components inhibits IL-12 production at the transcriptional level. Similarly, molecules expressed by *L. major* promastigotes and amastigotes can bind to macrophage CR3 and FcγRs, respectively, and reduce IL-12 production. The Th1 response that would kill the parasite is thus inhibited. Lastly, some parasites secrete hormone-like peptides that appear to downregulate the host immune response.

## F. Immunity to Fungi

### I. WHAT ARE FUNGI?

Fungi tend to be either unicellular and yeast-like (spherical), such as *Candida* species, or multicellular and filamentous, like *Aspergillus*. Some fungi live commensally on the topologically external surfaces of the body, while others live most of their lives in the soil as a mass (*mycelium*) of thread-like processes (*hyphae*). *Dimorphic* fungi adopt a yeast-like form at one stage in their life cycle and a hyphal form at another stage. Fungal cells have a cell wall like bacteria but also a cell membrane like mammalian cells. However, the fungal cell wall lacks the peptidoglycans, teichoic acids, and lipopolysaccharide components of the bacterial wall, and the main component of the fungal cell membrane is ergosterol rather than the cholesterol found in mammalian cell membranes.

Most fungal species are not harmful to healthy humans. However, immunocompromised individuals can suffer from acute infections that sometimes go on to become persistent. Such is the case with many *Candida* and *Aspergillus* species, which are part of the normal host flora but which can start to become invasive if immune surveillance by phagocytes fails. Species of soil-living filamentous fungi produce progeny in the form of *conidia* (spores), which can become airborne and thus inhaled by a host. Within the host, the conidia develop into either hyphae or other specialized forms better suited for host tissue invasion. However a pathogenic fungus accesses the body, its primary target tissue is usually the vascular system. Invasion of blood vessels by a growing fungus chokes off the blood supply to the host tissue, damaging or killing it. The exceptions are the *dermatophytes*, filamentous fungi that infect the skin, hair, and nails. These organisms, which include species of *Epidermophyton*, *Microsporum*, and *Trichophyton*, cannot penetrate the living, cellular tissue of a healthy host, and so are restricted to parts of the body that lack living cells, such as the keratinized outer layer of skin.

Diseases caused by fungal infections are called *mycoses*. Important fungal pathogens are *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Candida* species, *Aspergillus* species, *Cryptococcus neoformans*, and *Pneumocystis carinii* (Table 22-13). *Blastomycosis* occurs when conidia of the yeast-like fungus *B. dermatitidis* are inhaled. This organism replicates extracellularly to cause a pulmonary infection that spreads through the blood to the skin, bones, and male urogenital tract, but not the gut. In contrast, *H. capsulatum* is an intracellularly replicating yeast-like fungus that causes *histoplasmosis*. Inhaled microconidia develop into *Histoplasma* that take up residence preferentially in local respiratory macrophages. A progressive pulmonary disease resembling tuberculosis can spread to the secondary lymphoid organs, mucosae, gut, and adrenal glands. *Candida* species such as *C. albicans* and *C. tropicalis* lurk in the normal flora at the mucosae (but not in the skin) and cause disease only if these mucosal barriers are compromised. A deficiency of neutrophils in the host (*neutropenia*) leaves him or her especially vulnerable to *candidiasis*. Such *Candida* infections are usually fairly superficial in nature (such as vaginitis and cystitis) but can progress to infections of the eye, skin, and brain. Inhalation of spores of *Aspergillus* species causes a variety of diseases and can induce allergic responses. Conidia of three species, *A. fumigatus*, *A. flavus*, and *A. niger*, are particularly pathogenic for humans, causing invasive pulmonary infections that can be fatal if allowed to

Table 22-13 **Examples of Fungi and the Diseases They Cause**

| Organism | Disease |
|---|---|
| *Aspergillus* species, including *A. fumigatus*, *A. flavus*, *A. niger* | Airway and pulmonary infections and allergic reactions |
| *Blastomyces dermatitidis* | Blastomycosis (pulmonary infection) |
| *Candida* sp., *C. albicans*, *C. tropicalis* | Yeast infections, vaginitis, cystitis |
| *Cryptococcus neoformans* | Cryptococcosis (pulmonary infections and meningitis) |
| *Histoplasma capsulatum* | Histoplasmosis (TB-like disease) |
| *Pneumocystis carinii* | Pneumonia and severe lung damage |
| Dermatophytes, including *Epidermophtyon floccosum* | Infections of skin, nails, and hair |

entrench. Again, neutropenia is an aggravating factor. Clumps of filamentous *Aspergillus* readily colonize previously damaged airway tissues. *Aspergillus* species also produce *mycotoxins* that damage hepatocytes, macrophages, and CTLs. *C. neoformans* is a yeast-like fungus often present in pigeon droppings. When the unencapsulated spores of *Cryptococcus* are inhaled by a host, the parasite enters the lung and synthesizes a protective capsule that inhibits phagocytosis. If the infection becomes established, the result may be *cryptococcosis*, a syndrome of pulmonary infection accompanied by meningitis. *P. carinii* is a unicellular eukaryote that, although classified as a fungus, shares structural features with the protozoan parasites. *P. carinii* infection causes PCP (*P. carinii pneumonia*), a common feature in immunocompromised individuals (especially AIDS patients). Inhalation of *P. carinii* results in severe lung damage in hosts lacking cell-mediated immune responses. Spread of the infection to other tissues does not usually occur.

## II. EFFECTOR MECHANISMS

Effector mechanisms used against various fungi are summarized in Figure 22-14.

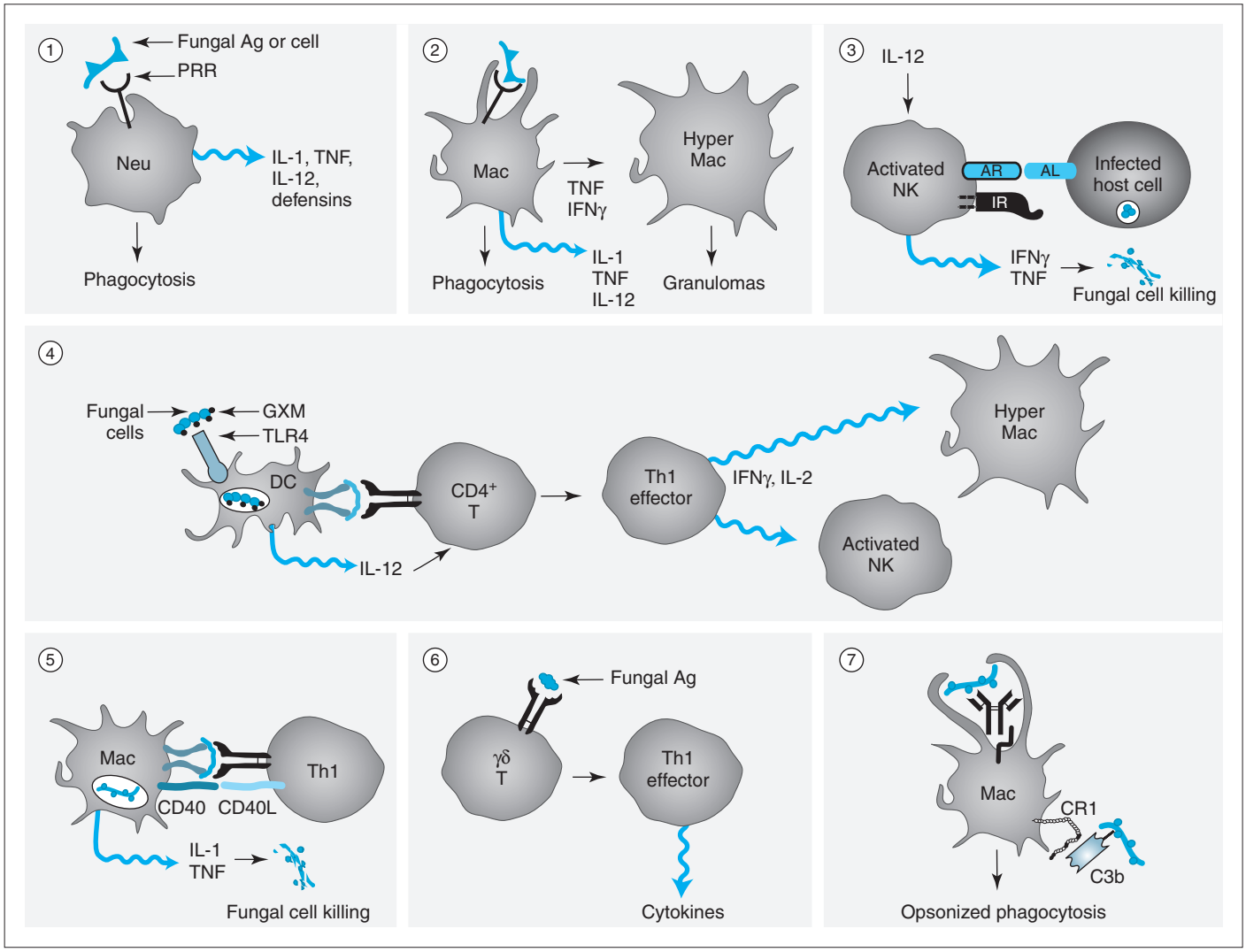



**Figure 22-14**

**Major Mechanisms of Immune Defense against Fungi**
(1) Neutrophils activated by PRR-mediated recognition of fungal PAMPs carry out phagocytosis and secrete cytokines. (2) Macrophages also carry out these functions plus can undergo hyperactivation and form granulomas to contain resistant fungi. (3) Activated NK cells kill fungi by secreting cytotoxic cytokines rather than by natural cytotoxicity. (4) Fungal TLR ligands such as GXM (glucuronooxylomannan) activate DCs that in turn initiate T cell activation and Th1 effector differentiation. These cells produce cytokines that stimulate macrophage hyperactivation and NK cell activation. (5) Infected macrophages that establish CD40-CD40L contacts with activated Th1 cells produce copious amounts of IL-1 and TNF that are directly toxic to fungal cells. (6) Mucosal γδ T cells activated by fungal products generate effectors that secrete additional cytokines supporting B cells. (7) Fungi coated in either anti-fungal antibody or C3b undergo opsonized phagocytosis by macrophages and neutrophils. Note that the structure of the fungal cell wall allows them to resist MAC lysis.

### i) Cell-Mediated Defense

As mentioned previously, most fungi do not establish infections in humans with competent innate and adaptive immune responses. Cell-mediated innate immunity is the primary means by which fungus infections are controlled. Neutrophils and macrophages both carry out vigorous phagocytosis and produce powerful anti-fungal defensins. These defensins induce an osmotic imbalance in pathogens such as *Candida* and *Cryptococcus* that kills them. Neutrophils and macrophages also secrete copious quantities of IL-1, IL-12, and TNF. As we have seen, IL-12 stimulation activates NK cells that contribute to fungal cell killing via cytokine secretion (rather than natural cytotoxicity). IFNγ produced first by activated NK cells and later by activated Th1 cells also hyperactivates macrophages, which can initiate granuloma formation.

Interestingly, a fungus present in its unicellular yeast-like form tends to provoke a protective Th1 response, whereas its hyphal form tends to induce a non-protective Th2 response. There is some evidence that either distinct subsets of DCs, or distinct receptors on DCs, respond to the two different fungal morphologies. These DCs then proceed with phagocytosis and antigen processing and presentation, and influencing Th1/Th2 differentiation in the direction best suited to eliminate the particular form of the fungus present. The identity of the receptors driving this recognition is not yet clear, but it has been shown that, much like it binds to the LPS of bacteria, TLR4 can recognize and bind to the polysaccharide *glucuronoxylomannan* (GXM) present in the capsule of *C. neoformans*. In addition, in one model of *Candida* infection, TLR4-deficient mice had a higher pathogen burden than wild-type controls.

The Th1 response induced by exposure to airborne fungal spores, or invasion by skin or mucosal fungal flora that have a yeast-like form, is mediated by cells producing copious quantities of IL-2 and IFNγ. Accordingly, IL-12 production has an anti-fungal effect, as evidenced by the fact that mice deficient for the p40 subunit of IL-12 are highly susceptible to *C. albicans* infection. As mentioned previously, neutrophils, along with macrophages, appear to be the key producers of IL-12 during fungal infections. Thus, individuals with neutropenia show enhanced susceptibility to attack by these pathogens. The exception appears to be *P. carinii* infection. In animal models, it is macrophage/monocyte production of TNF and IL-1 that is most important for defense. The production of these cytokines depends on CD40L contacts supplied by activated T cells.

Th2 responses are comparatively rare during infections with yeast-like fungi. Those patients that respond with Th2 responses in place of Th1 responses when attacked by a yeast-like form show decreased resistance to the fungus. Such a bias appears in patients suffering from chronic mucosal *C. albicans* infections. While DTH responses in the skin counteract fungal invasion, the presence of Th2-associated antibodies such as IgE and IgG4 (in the absence of a DTH response) can actually predispose patients to recurrent fungal infections.

γδ T cells play a significant role in anti-fungal defense at the mucosae. Mice deficient for γδ T cells show enhanced susceptibility to *C. albicans* infections. In wild-type mice, polyclonal expansion of γδ T cells in the gastric mucosa occurs upon oral exposure to *C. albicans*. Protein antigens isolated from certain fungi can be used *in vitro* to stimulate human γδ T cells to produce factors promoting B cell differentiation.

### ii) Humoral Defense

Other than the secretory IgA that defends the mucosae, antibodies are thought to contribute in only a limited way to defense against fungi. Antibody-mediated opsonization may promote phagocytosis, and thus contribute to the presentation of fungal antigens that activates Th1 cells. Mice deficient for B cells show a deficit in cell-mediated immunity that allows the establishment of infections with *Candida* species and *P. carinii*.

### iii) Complement

While fungal cells can activate the complement cascade, they are generally resistant to complement-mediated lysis. However, they are subject to phagocytosis when opsonized by complement products. Fungi also express analogues of complement receptors that facilitate adherence to host cells and may also promote phagocytosis. Pro-inflammatory cytokines induced by products of complement activation also contribute to anti-fungal defense.

## III. EVASION STRATEGIES

As mentioned previously, many fungi adopt different forms at different stages in their life cycles, making immune defense necessarily more complex. The structure of the fungal cell wall and membrane means that fungi generally can avoid complement-mediated lysis. In addition, many fungi have developed strategies to offset the effector actions of neutrophils, macrophages, CTLs, and NK cells (Table 22-14).

Table 22-14 **Evasion of the Immune System by Fungi**

| Immune System Element Thwarted | Fungal Mechanism |
|---|---|
| PRR recognition | Have no LPS or peptidoglycan in cell wall |
| Specificity of T and B cells | Have a multi-stage life cycle |
| Complement | Block access to cell membrane via cell wall |
| Phagocytosis | Block phagocytosis via polysaccharide capsule |
| T and B cell function | Induce immune deviation to Th2<br>Block NF-κB activation<br>Increase NO production to decrease lymphocyte proliferation<br>Block phagocytosis<br>Inhibit neutrophil migration<br>Decrease IL-12 and B7 expression by monocytes<br>Activate regulatory T cells via polysaccharide capsule component<br>Produce melanin to decrease Th1 and Th2 responses<br>Block TNF production |

Many fungi produce toxins and other molecules that have immunosuppressive effects. Some appear to mediate immune deviation, biasing the host's immune reactions to ineffective Th2 responses at the expense of Th1 responses. Others have more direct inhibitory effects. For example, *gliotoxin*, a metabolite produced by *A. fumigatus*, inhibits the activation of NF-κB in stimulated T and B cells, blocking the transcription of genes needed for differentiation. A molecule called *protein 43* acts in an unknown way to suppress immune responses during murine *Candida* infections. *H. capsulatum* infection in mice results in NO production that downregulates lymphocyte proliferation. The GXM polysaccharide present in the capsule of *C. neoformans* spores not only blocks phagocytosis and inhibits neutrophil migration but also has downregulatory effects on adaptive immunity. In its free form, GMX blocks production of IL-12 by monocytes, downregulates B7 expression on macrophages, and activates regulatory T cells. In addition, melanin produced by *C. neoformans* inhibits both Th1 and Th2 responses in mice. The BAD1 molecule of *B. dermatitidis* binds to CR3 on macrophages and blocks the production of TNF by these cells.

## G. The Mysterious Prions

For decades, scientists were puzzled by a number of related neurodegenerative diseases found in both humans and animals. These diseases came to be known as *spongiform encephalopathies* (SE) because they all caused CNS lesions that rendered the brain "sponge-like." Spongiform encephalopathies, which are invariably fatal, include variant Creutzfeldt-Jakob disease (vCJD) and kuru in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE or "mad cow disease") in cattle. Over the years, it was found that each of these diseases could be transmitted to experimental animals by injecting brain extracts from patients or animals that had died of SE. Indeed, D. Carleton Gajdusek was awarded a Nobel Prize in 1976 for his discovery in the early 1960s that kuru in humans represented a new form of infectious disease. However, while an infectious etiology had been clearly established, the actual agents involved remained mysterious. Analysis of the scrapie agent revealed it to be extremely resistant to heat, formalin, and both UV- and ionizing irradiation—all treatments that normally destroy infectious pathogens. Furthermore, scrapie, kuru, and vCJD did not provoke any of the normal immune responses expected during an infection. An infectious etiology was also difficult to reconcile with the earlier identification in the 1930s of a rare, familial form of vCJD that presumably had a genetic etiology. Finally, an SE would occasionally arise in a sporadic manner in a patient or animal with no history of familial or infectious transmission.

In the 1970s, Stanley Prusiner of the University of California at San Francisco attempted to purify the "scrapie agent" to determine its molecular nature. Surprisingly, protocols that destroyed nucleic acids had no effect on scrapie infectivity, while procedures known to destroy proteins reduced its infectious strength. By 1982, Prusiner concluded that the agent responsible for scrapie infectivity was a protein devoid of any nucleic acid, an unprecedented discovery in biology. At this point, he coined the term "prion" to identify this novel type of pathogen that was both proteinaceous and infectious. The scrapie agent was therefore denoted as $PrP^{sc}$ (*prion protein, scrapie*). Further work by Prusiner established that $PrP^{sc}$ was actually a conformational isomer of a normal glycoprotein found on the surfaces of many mammalian cells. This normal glycoprotein was denoted $PrP^{c}$ (*prion protein, cellular*). It turns out that, upon exposure to a $PrP^{sc}$ molecule, a $PrP^{c}$ molecule undergoes a dramatic change in conformation. An α-helical region of the native $PrP^{c}$ protein spontaneously re-folds into the β-pleated sheet structure characteristic of $PrP^{sc}$. This refolding process appears to be facilitated by an as-yet-unidentified co-factor (perhaps a membrane lipid). It is also unclear exactly where the conversion occurs, although there is some evidence pointing to the minute infoldings of the plasma membrane generated during pinocytosis (called *caveolae*) as the site. In any case, when $PrP^{sc}$ is introduced into a fresh host, it acts as a template for the refolding of existing host $PrP^{c}$ molecules into additional copies of $PrP^{sc}$. In other words, the disease-causing prion can effectively "replicate" itself in a new host and a mass conversion of the host's $PrP^{c}$ molecules to the $PrP^{sc}$ conformation occurs (Fig. 22-15). Unlike $PrP^{c}$, the misfolded $PrP^{sc}$ has profoundly altered physicochemical properties. When the $PrP^{sc}$ protein invades cells of the CNS, it induces brain degeneration that is manifested as the clinical signs of SE. Intriguingly, no other part of the body appears to be affected by the presence of $PrP^{sc}$. For his discovery of prions and his investigations into the mechanisms by which they cause disease, Dr. Prusiner was awarded a Nobel Prize in Physiology or Medicine in 1997.

We emphasize that, in this infectious form of prion disease, there is no mutation of the $PrP^{c}$ gene of the host, and no change in the amino acid sequence of the affected $PrP^{c}$ proteins; the disorder is purely one of protein misfolding. Interestingly, it appears that misfolding of native proteins may be implicated in other neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and frontotemporal dementia. Other forms of prion disease are inherited rather than infectious and do involve a mutation of the host's $PrP^{c}$ gene. More than 20 such mutations have been identified, some of which may account for the sporadic cases of prion disease that have been reported over the years. Once a mutated $PrP^{c}$ gene arises *de novo* in one individual, the pathogenic gene is passed down to his or her offspring.

The BSE epidemic in Great Britain in the 1990s was likely a case of infectious prion disease caused by the feeding of scrapie-infected sheep offal to cattle. A small number of humans who consumed beef processed from these cattle then acquired the $PrP^{sc}$ protein and became ill with vCJD. The use of sheep offal as animal feed is now banned, but 1 million cattle were thought to have been infected with prions before this restriction came into place. Infectious prions are also responsible for the human disease kuru. Kuru was found almost exclusively among the Fore people of New Guinea, a tribe that used to routinely engage in ritualistic cannibalism. This practice included eating the brain tissue of dead relatives, some of whom had died of kuru. At the urging of missionaries,

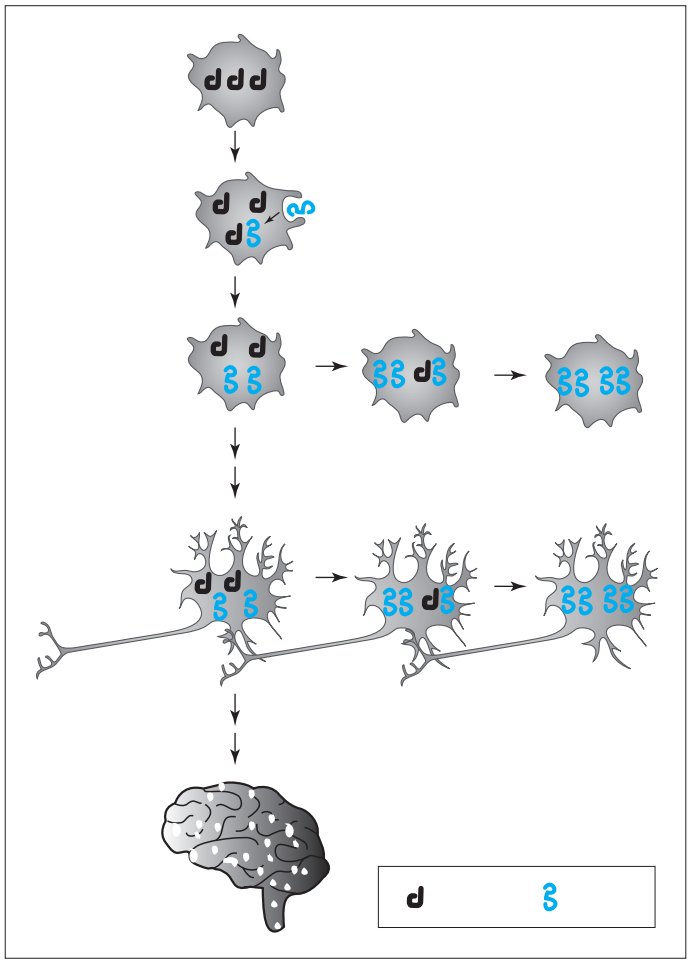


Figure 22-15

**Infectious Prion Disease**
A phagocyte in a healthy animal contains normally folded $PrP^c$ molecules. Disease is initiated when the healthy animal ingests an abnormally folded prion $PrP^{sc}$ molecule in contaminated food. A phagocyte takes up the $PrP^{sc}$ molecule, which serves as a template for the misfolding of the cell's normal $PrP^c$ molecules. Gradually, more and more normal $PrP^c$ molecules are converted into $PrP^{sc}$ molecules but the converted phagocyte does not die. Rather, it conveys the $PrP^{sc}$ molecules to neurons in the CNS that are killed upon uptake of $PrP^{sc}$ and conversion of neuron $PrP^c$ molecules. Massive neuronal death causes the "spongy brain" and other symptoms of spongiform encephalopathy (SE).

the practice of cannibalism ended in the late 1950s, so that the incidence of kuru has declined steadily since that time.

Prion infection destroys the brain without inducing either a humoral or cell-mediated adaptive response. The host's T cells are usually tolerant to the $PrP^{sc}$ protein, as it is merely a naturally occurring host protein with modified secondary structure. Thus, almost all peptides generated from newly formed $PrP^{sc}$ will be seen as "self" peptides. By extension, in the absence of the activation of prion-specific T cells, no Td humoral response can be mounted. Furthermore, although the "foreign" conformation of $PrP^{sc}$ might be recognized by the BCR of a B cell, the antigen itself cannot act as a Ti immunogen as it has neither the large size or multi-valency needed to stimulate B cells directly.

Despite the preceding, work in experimental animals has shown that the $PrP^c$ protein can be immunogenic. In so-called $PrP^{0/0}$ mice, in which the gene encoding $PrP^c$ has been knocked out, the natural tolerance to the protein is absent and a normal, $PrP^c$-specific humoral response is seen after immunization with murine $PrP^c$ in adjuvant. In another experiment, $PrP^{0/0}$ mice were immunized with plasmids expressing DNA (see Ch.23) encoding human prion proteins, inducing production of mouse anti-human $PrP^c$ antibodies. Similarly, chickens immunized with either human or bovine $PrP^c$ coupled to the carrier KLH generate antibodies directed against human or bovine $PrP^c$. Such anti-$PrP^c$ antibody preparations are useful for both the study of prions and as potential tools for the diagnosis, prevention, and treatment of prion diseases.

In view of the natural tolerance to $PrP^c$ discussed previously, one might assume that the immune system is neutral with respect to prion diseases. However, it appears that the immune system may actually promote the progress of prion disease both directly and indirectly. In a natural setting, prion infection occurs by a peripheral route, usually oral. Leukocytes are among the first cells to take up the prions, but the "replication" of the prions via $PrP^c$ conversion causes no harm to these cells, in contrast to the severe damage done to neurons. However, leukocytes are apparently responsible for the subsequent introduction of prions into the CNS. In addition, it seems that the FDC in the lymphoid follicles is required for the replication of prions that precedes their spread into the nervous system. The mechanism remains obscure but is an absolute requirement for prion disease progression. There is also evidence that the activation of specific complement components plays a role in the initial trapping of prions in the lymphoid organs soon after infection. This too may relate to FDC function, since C3d/C4b-opsonized antigens interact with CR2/CR1 complement receptors on FDC and may therefore be important for the intracellular accumulation of prions in the FDC. Other studies have suggested that macrophages and DCs can be involved in prion disease, and that a microbial or viral pathogen may sometimes act as a triggering co-factor.

Indirect support for the involvement of the immune system in prion pathogenesis comes from studies of mouse models of scrapie infection. In these experiments, susceptibility to prion disease correlated with the functional status of the immune system. For example, while mitogenic stimulation of lymphoid cells enhanced prion disease susceptibility, lymphoid suppression achieved by either corticosteroid administration or splenectomy reduced it. In addition, studies of mice assessed at various time points after birth showed that increasing susceptibility to prion infection correlated with the progressive maturation of the immune system. Lastly, and perhaps most strikingly, *scid* mice (which cannot mount either T or B cell-mediated responses) were resistant to infection by the BSE or scrapie agents, while immunologically reconstituted *scid* mice were not. A more complete understanding of prion infection and disease processes will be critical to development of prevention strategies and therapies for these fascinating but deadly disorders.

This brings us to the end of our description of natural immune defense against pathogens. We move now to a discussion of "manufactured" immunity to pathogens, created by the techniques of vaccination.

## SUMMARY

Immune responses have evolved to combat the five major types of pathogens: extracellular bacteria, intracellular bacteria, viruses, parasites, and fungi. For all types of pathogens, the mechanisms of innate immunity offer an immediate response that either foils the establishment of infection or slows the infection down until adaptive immune mechanisms can target the pathogen more effectively. When an adaptive immune response is activated, the elements that will be most effective depend on whether the pathogen is extracellular or intracellular. Extracellular entities that are relatively small, such as extracellular bacteria, virus particles, protozoan parasites, and some fungi, can be targeted by antibody and then cleared effectively by antibody- and complement-mediated mechanisms that involve either direct lysis or phagocytic destruction. Protozoan worms are also subject to antibody assault because they are extracellular; however, these organisms are too large to be subsequently engulfed. Instead, specialized antibody isotypes such as IgA and IgE are produced to ensure that the worm does not become anchored in the host. In addition, mediators released by degranulating mast cells and eosinophils act on worm tissues to degrade them. Intracellular bacteria and replicating viruses, which are found inside host cells and cannot be targeted by antibody, must be eliminated by cytolytic mechanisms mediated by CTLs, NK cells, NKT cells, and $\gamma\delta$ T cells. A successful immune response against a given pathogen thus depends on cells and cytokines of the innate response inducing Th responses of the appropriate subtype, with Th1 responses being required for cell-mediated immunity against internal threats, and Th2 responses being needed for humoral responses against external threats. In the quest for continued survival, many pathogens have evolved complex evasion strategies intended to compromise the success of the immune response invoked.


## Selected Reading List


Abel L. and Dessein A. J. (1997) The impact of host genetics on susceptibility to human infectious diseases. *Current Opinion in Immunology* **9**, 509–516.

Bachmann M. and Kopf M. (2002) Balancing protective immunity and immunopathology. *Current Opinion in Immunology* **14**, 413–419.

Bangham C. R. (2000) The immune response to HTLV-I. *Current Opinion in Immunology* **12**, 397–402.

Barry M. and McFadden G. (1998) Apoptosis regulators from DNA viruses. *Current Opinion in Immunology* **10**, 422–430.

Basta S. and Bennink J. R. (2003) A survival game of hide and seek: cytomegaloviruses and MHC class I antigen presentation pathways. *Viral Immunology* **16**, 231–242.

Bertoletti A. and Maini M. K. (2000) Protection or damage: a dual role for the virus-specific cytotoxic T lymphocyte response in hepatitis B and C infection? *Current Opinion in Immunology* **12**, 403–408.

CDC Public Health Emergency Preparedness and Response (http: //www.bt.cdc.gov/index.asp).

Cohen J. I. (1999) The biology of Epstein-Barr virus: lessons learned from the virus and the host. *Current Opinion in Immunology* **11**, 365–370.

Cossart P. and Bierne H. (2001) The use of host cell machinery in the pathogenesis of *Listeria monocytogenes*. *Current Opinion in Immunology* **13**, 96–103.

Cuconati A. and White E. (2002) Viral homologs of BCL-2: role of apoptosis in the regulation of virus infection. *Genes and Development* **16**, 2465–2478.

Dimopoulous G., Muller H.-M., Levashina E. A. and Kafatos F. C. (2001) Innate immune defense against malaria infection in the mosquito. *Current Opinion in Immunology* **13**, 79–88.

Dussurget O., Pizarro-Cerda J. and Cossart P. (2004) Molecular determinants of *Listeria monocytogenes* virulence. *Annual Review of Microbiology* **58**, 587–610.

Edelson B. T. and Unanue E. R. (2000) Immunity to *Listeria* infection. *Current Opinion in Immunology* **12**, 425–431.

Finkelman F. D., Wynn T. A., Donaldson D. D. and Urban Jr. J. F. (1999) The role of IL-13 in helminth-induced inflammation and protective immunity against nematode infections. *Current Opinion in Immunology* **11**, 420–426.

Flynn J. L. and Ernst J. D. (2000) Immune responses in tuberculosis. *Current Opinion in Immunology* **12**, 432–436.

Flynn J. L. and Chan J. (2001) Immunology of tuberculosis. *Annual Review of Immunology* **19**, 93–129.

Friedlander A. (2001) Microbiology: tackling anthrax. *Nature* **414**, 160–161.

Good M. F. (1999) Immune effector mechanisms in malaria. *Current Opinion in Immunology* **11**, 412–419.

Grandvaux N., tenOever B., Servant M. and Hiscott J. (2002) The interferon antiviral response: from viral invasion to evasion. *Current Opinion in Infectious Disease* **15**, 259–267.

Grewal I. S., Borrow P., Pamer E. G., Oldstone M. B. and Flavell R. A. (1997) The CD40-CD154 system in anti-infective host defense. *Current Opinion in Immunology* **9**, 491–497.

Hay S. and Kannourakis G. (2002) A time to kill: viral manipulation of the cell death program. *Journal of General Virology* **83**, 1547–1564.

Hengel H. and Koszinowski U. H. (1997) Interference with antigen processing by viruses. *Current Opinion in Immunology* **9**, 470–476.

Hilleman M.R. (2004) Strategies and mechanisms for host and pathogen survival in acute and persistent viral infections. *Proceedings of the National Academy of Sciences USA* **101**(Suppl. 2), 14560–14566.

Hunter C. A. and Reiner S. L. (2000) Cytokines and T cells in host defence. *Current Opinion in Immunology* **12**, 413–418.

Kemp A. and Bjorksten B. (2003) Immune deviation and the hygiene hypothesis: a review of the epidemiological evidence. *Pediatric Allergy and Immunology* **14**, 74–80.

Kerksiek K. and Pamer E. G. (1999) T cell responses to bacterial infection. *Current Opinion in Immunology* **11**, 400–405.

Klein M., Kaeser P., Schwarz P., Weyd H., Xenarios I. *et al.* (2001) Complement facilitates early prion pathogenesis. *Nature Medicine* **4**, 488–492.

Lindahl G., Sjobring U. and Johnsson E. (2000) Human complement regulators: a major target for pathogenic microorganisms. *Current Opinion in Immunology* **12**, 44–51.

Lingelbach K., Kirk K., Rogerson S., Langhorne J. *et al.* (2004) Molecular approaches to malaria. *Molecular Microbiology* **5**, 575–587.

Louis J., Himmelrich H., Parra-Lopez C., Tacchini-Cottier F. and Launois P. (1998) Regulation of protective immunity against *Leishmania major* in mice. *Current Opinion in Immunology* **10**, 459–464.

MacDonald A., Araujo M. and Pearce E. (2001) Immunology of parasitic helminth infections. *Infection and Immunity* **70**, 427–433.

Maizels R. and Yazdanbakhsh M. (2003) Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology* **3**, 733–744.

Malaviya R. and Abraham S. (2001) Mast cell modulation of immune responses to bacteria. *Immunological Reviews* **179**, 16–24.

Mansour M. and Levitz S. (2002) Interactions of fungi with phagocytes. *Current Opinion in Microbiology* **5**, 359–365.

McGuinness D., Dehal P., and Pleass R. (2003) Pattern recognition molecules and innate immunity to parasites. *Trends in Parasitology* **19**, 312–319.

Mims C. (1982) "The Pathogenesis of Infectious Disease," 3rd edn. Academic Press, London.

Moll H. (2003) Dendritic cells and host resistance to infection. *Cell Microbiology* **5**, 493–500.

Montagna L., Ivanov M. and Bliska J. (2001) Identification of residues in the N-terminal domain of the *Yersinia* tyrosine phosphatase that are critical for substrate recognition. *Journal of Biological Chemistry* **276**, 5005–5011.

Moorthy V. and Hill A. (2002) Malaria vaccines. *British Medical Bulletin* **62**, 59–72.

Morgan B. P. (1999) Regulation of the complement membrane attack pathway. *Critical Reviews in Immunology* **19**, 173–198.

Mosser D. M. and Karp C. (1999) Receptor mediated subversion of macrophage cytokine production by intracellular pathogens. *Current Opinion in Immunology* **11**, 406–411.

Neild A. L. and Roy C. R. (2004) Immunity to vacuolar pathogens: what can we learn from *Legionella? Cell Microbiology* **6**, 1011–1018.

Neutra M. R., Pringault E. and Kraehenbuhl J.-P. (1996) Antigen sampling across epithelial barriers and induction of mucosal immune responses. *Annual Review of Immunology* **14**, 275–300.

Palucka K. and Banchereau J. (2002) How dendritic cells and microbes interact to elicit or subvert protective immune responses. *Current Opinion in Immunology* **14**, 420–431.

Pieters J. (2001) Evasion of host cell defense mechanisms by pathogenic bacteria. *Current Opinion in Immunology* **13**, 37–44.

Plebanski M. and Hill A. V. (2000) The immunology of malaria infection. *Current Opinion in Immunology* **12**, 437–441.

Ploegh H. (1998) Viral strategies of immune evasion. *Science* **280**, 248–253.

Reddehase M. (2000) The immunogenicity of human and murine cytomegaloviruses. *Current Opinion in Immunology* **12**, 390–396.

Reis e Sousa C., Sher A. and Kaye P. (1999) The role of dendritic cells in the induction and regulation of immunity to microbial infection. *Current Opinion in Immunology* **11**, 392–399.

Rescigno M. (2002) Dendritic cells and the complexity of microbial infection. *Trends in Microbiology* **10**, 425–431.

Roeder A., Kirschning C., Rupec R., Schaller M. and Korting H. (2004) Toll-like receptors and innate antifungal responses. *Trends in Microbiology* **12**, 44–49.

Romani L. (1997) The T cell response against fungal infections. *Current Opinion in Immunology* **9**, 484–490.

Romani L., Bistoni F. and Puccetti P. (2002) Fungi, dendritic cells and receptors: a host perspective of fungal virulence. *Trends in Microbiology* **10**, 508–514.

Roulston A,. Marcellus R. and Branton P. (1999) Viruses and apoptosis. *Annual Review of Microbiology* **53**, 577–628.

Sansonetti P. J. and Phalipon A. (1999) M cells as ports of entry for enteroinvasive pathogens: mechanisms of interaction, consequences for the disease process. *Seminars in Immunology* **11**, 193–203.

Schluger N. and Rom W. (1997) Early responses to infection: chemokines as mediators of inflammation. *Currrent Opinion in Immunology* **9**, 504–508.

Scholtissek C. (2002) Pandemic influenza: antigenic shift. In Potter C. W., ed. "Influenza." Elsevier Science B.V., Amsterdam, 87–100.

Segal S. and Hill A.V. (2003) Genetic susceptibility to infectious disease. *Trends in Microbiology* **11**, 445–448.

Shen H., Tato C. M. and Fan X. (1998) *Listeria monocytogenes* as a probe to study cell-mediated immunity. *Current Opinion in Immunology* **10**, 450–458.

Spriggs M. K. (1999) Shared resources between the neural and immune systems: semaphorins join the ranks. *Current Opinion in Immunology* **11**, 387–391.

Stenger S. and Modlin R. L. (1998) Cytotoxic T cell responses to intracellular pathogens. *Current Opinion in Immunology* **10**, 471–477.

Szabo S., Sullivan B., Peng S. and Glimcher L. (2003) Molecular mechanisms regulating Th1 immune responses. *Annual Review of Immunology* **21**, 713–758.

Szomolanyi-Tsuda E. and Welsh R. M. (1998) T-cell-independent antiviral antibody responses. *Current Opinion in Immunology* **10**, 431–435.

Tatis N., Sinnathamby G. and Eisenlohr L. (2004) Vaccinia virus as a tool for immunologic studies. *Methods in Molecular Biology* **269**, 267–288.

Taylor G., Feng C. and Sher A. (2004) p47 GTPases: regulators of immunity to intracellular pathogens. *Nature Reviews Immunology* **4**, 100–109.

Tortora G., Funke B. and Case C. (2004) "Microbiology: An Introduction," 7th edn. Benjamin Cummings, San Francisco.

Tortorella D., Gewurz B. E., Furman M. H., Schust D. J. and Ploegh H. L. (2000) Viral subversion of the immune system. *Annual Review of Immunology* **18**, 861–926.

Turner M.W. (2003) The role of mannose-binding lectin in health and disease. *Molecular Immunology* **40**, 423–429.

Urban B. and Robers D. (2002) Malaria, monocytes, macrophages and myeloid dendritic cells: sticking of infected erythrocytes switches off host cells. *Current Opinion in Immunology* **14**, 458–465.

Wold W. S., Doronin K., Toth K., Kuppuswamy M., Lichtenstien D. *et al.* (1999) Immune responses to adenoviruses: viral evasion mechanisms and their implications for the clinic. *Current Opinion in Immunology* **11**, 380–386.

Wurzner R. (1999) Evasion of pathogen by avoiding recognition or eradication by complement, in part via molecular mimicry. *Molecular Immunology* **36**, 249–260.

Yanagi Y. (2001) The cellular receptor for measles virus—elusive no more. *Reviews in Medical Virology* **11**, 149–156.