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Immunity to Infection

Chapter 13

WHAT'S IN THIS CHAPTER?

If one way be better than another, that you may be sure is Nature's way.

Aristotle

Infectious diseases lead to about 14 million human deaths annually. These maladies are caused by six types of pathogens: **extracellular bacteria, intracellular bacteria, viruses, parasites, fungi** and **prions**. Bacteria are microscopic, single-celled, prokaryotic organisms. Extracellular bacteria do not have to enter host cells to reproduce, whereas intracellular bacteria do. Viruses are submicroscopic, acellular particles that consist of a protein coat surrounding an RNA or DNA genome. To propagate, a virus must enter a host cell and exploit its protein synthesis machinery. Parasites are eukaryotic organisms that take advantage of a host for habitat and nutrition at some point in their life cycles. Parasites often damage a host but kill it only slowly. Parasites may be tiny, single-celled *protozoans;* large, multicellular *helminth worms;* or arthropod *ectoparasites*. Fungi are eukaryotic organisms that can exist comfortably outside a host but will invade and colonize that host if given the opportunity. Fungi may be single-celled or multicellular. Prions are infectious proteins that cause neurological disease by altering normal proteins in the brain of the infected host.

Infection occurs when an organism successfully avoids innate defense and colonizes a niche in the body. What follows is a biological "horse race" in which the pathogen tries to replicate and expand its niche, while the immune system tries to eliminate the pathogen (or at least confine it). Only if the replication of the pathogen results in detectable clinical damage does the host experience "disease." Microbial **toxins** released by a pathogen can cause disease even in the absence of widespread colonization. **Immunopathic damage** may occur if host tissues are unintentionally injured by the immune response as it strives to destroy a pathogen. As detailed in Sections A–G that follow, the innate and adaptive effector mechanisms best suited to countering a particular pathogen are determined by the invader's lifestyle and mode of replication.

MAK: Primer to the Immune Response. http://dx.doi.org/10.1016/B978-0-12-385245-8.00013-3

NOTE: Although patients go to hospitals to be cured, about 5% of them will acquire an infection after admission, and about 5% of these individuals will die of these infections. Indeed, in the USA and Europe, *nosocomial* (hospital-acquired) infections are the sixth leading cause of death. In both jurisdictions, billions are spent every year to deal with this problem, even though an estimated one-third of these infections are preventable. Gram-negative bacteria are often the culprits, and pneumonia is the most common life-threatening clinical consequence. Infections of the bloodstream, urinary tract, and surgical sites are also frequent. Individuals who are immunosuppressed are particularly vulnerable to hospital-acquired infections and may succumb to organisms that would otherwise be successfully repelled. Such individuals include cancer patients treated with chemotherapy or radiation, and transplant patients taking medications designed to suppress their immune systems and prevent transplant rejection.

A. General Features of Host–Pathogen Encounters

Most of the mechanisms of innate defense described in detail in Chapter 3 can help the host combat any type of pathogen. The first obstacles encountered by an invader are the intact skin and mucosae. Pathogens are prevented from gaining a firm foothold on the skin by the toughness and routine shedding of the keratin layers protecting the epidermis, and also by having to compete with commensal microorganisms. Pathogens ingested into the gut or inhaled into the respiratory tract are trapped by mucus or succumb to microbicidal molecules in the body secretions or to the low pH and hydrolases of the gut. However, a breach of the skin or mucosae may allow a pathogen access to subepithelial tissues. Barrier penetration may also occur in individuals whose immune systems have been compromised by either disease or therapeutic immunosuppression. These lapses in immune defense may allow **opportunistic pathogens**, which are normally harmless to a healthy individual, to cause disease. In contrast, **invasive pathogens** can enter the body even when surface defenses are intact. Invasive organisms assaulting the mucosae frequently gain access via the M cells of the FAE or by binding to host cell surface molecules that initiate receptor-mediated internalization.

Recall that FAE is a region of follicle-associated epithelium in a body tract mucosa as described in Chapter 12 and illustrated in Figure 12-2.

A pathogen that penetrates the skin or mucosae triggers the flooding of the site with acute phase proteins, pro-inflammatory cytokines such as IL-1 and TNF, and complement components. Coating of the pathogen by C3b or MBL facilitates its elimination by the alternative or lectin complement cascades, respectively. At a cellular level, general innate defense is mediated by the PRRs of resident DCs, neutrophils and other granulocytes, macrophages, NK cells, γδ T cells and NKT cells. These PRRs include TLRs, NLRs, RLRs, CLRs, scavenger receptors, and cell-bound collectins, as well as the antigen recognition receptors of NK, NKT and γδ T cells. In addition, soluble collectins in the extracellular matrix that have bound to pathogens or their products may activate complement or stimulate phagocytosis.

Recall that several classes of PRRs expressed by innate leukocytes were illustrated in Figure 3-4 and their features summarized in Table 3-2.

NOTE: Recent research has revealed a prominent antipathogen role for the inflammasomes generated following NLR engagement. As described in Chapter 3 and illustrated in Figure 3-5, the engagement of the NLRs NLRP1, NLRP3 or NLRC4 triggers the formation of the NLRP1, NLRP3 or NLRC4 inflammasome, respectively. For example, the NLRP3 inflammasome is activated in response to DAMPs such as host-derived uric acid or cholesterol crystals, or PAMPs derived from extracellular bacteria such as *Streptococcus pneumoniae* and *Yersinia enterocolitica*, or from intracellular bacteria such as *Listeria monocytogenes, Bordatella pertussis* and *Legionella pneumophila.* Viral PAMPs (such as those derived from influenza virus), parasite PAMPs (such as those derived from *Schistosoma mansoni* or *Plasmodia falciparum*), or fungal PAMPs (such as those derived from *Candida albicans*) may also induce NLRP3 formation. The PAMPs in these cases include bacterial toxins, viral ssRNA or dsRNA, fungal cell wall components, or parasite egg antigens. NLRC4 inflammasomes also respond to PAMPs from *Salmonella*, *Legionella* or *Pseudomonas* species. NLRP1 inflammasomes are activated by a toxin of *Bacillus anthracis* and have been implicated in combatting some herpesvirus infections.

Recall that inflammasome assembly results in the processing and activation of the key pro-inflammatory cytokines IL-1 and IL-18 (see Ch. 3).

In a site of pathogen attack, local leukocytes activated by PRR engagement attempt to eliminate the pathogen or infected cells by clathrin-mediated endocytosis or phagocytosis, secretion of cytotoxic cytokines, or perforin/granzyme-mediated cytotoxicity. These cells also contribute toxic NO and ROIs to the extracellular milieu. Chemokines produced in the ensuing inflammatory response draw neutrophils and other leukocytes from the circulation into the area of infection to assist in the fight. If a pathogen enters the blood, innate defense falls to monocytes and neutrophils in the circulation. Organisms that reach the liver or the spleen are confronted by resident macrophages.

As the innate response proceeds, local DCs that have matured due to exposure to pathogen components become competent to present pathogen-derived pMHCs to naïve T cells, triggering the adaptive response. In many cases, this T cell activation and subsequent B cell activation will take place in inductive sites in the MALT or in the SALT, and the effector cells generated will migrate to effector sites at the body's portals to fight the pathogen. A systemic immune response will soon follow if mature DCs bearing pathogen antigens migrate to lymphoid follicles in the draining lymph node or spleen and activate naïve T and B cells in these locations.

B. Immunity to Extracellular Bacteria

I. Disease Mechanisms

Extracellular bacteria attempting to establish an infection tend to accumulate in interstitial regions in connective tissues; in the lumens of the respiratory, urogenital and gastrointestinal tracts; and in the blood. These organisms often secrete proteins that penetrate or enzymatically cleave components of the mucosal epithelium, allowing access to underlying tissues **(Plate 13-1)**. A wide variety of extracellular bacteria enter the M cells in the FAE, whereas others exploit surface receptors on other host cell types. Examples of diseases caused by infections with extracellular bacteria are given in **Table 13-1**.

Many disease symptoms caused by extracellular bacteria can be attributed to their toxins. **Exotoxins** are toxic proteins actively secreted by either *Gram-positive* or *Gram-negative* bacteria. **Gram-positive bacteria** have cell walls containing a thick layer of peptidoglycan that is colored purple after Gram staining. **Gram-negative bacteria** have cell walls containing a thin layer of peptidoglycan plus LPS that is colored red after Gram staining. **Endotoxins** are the lipid portions of the LPS molecules embedded in the walls of Gram-negative bacteria. Endotoxins are not

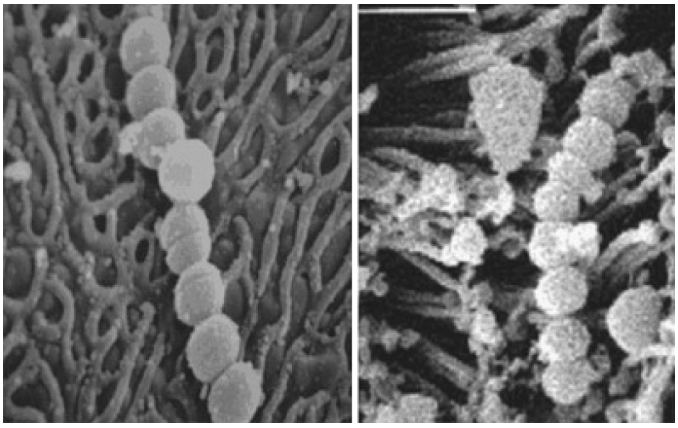

Plate 13-1
Attack by Extracellular Bacteria

Scanning electron micrograph showing *Streptococcus* cells (left panel) attached to the epithelial cells forming the surface of the lingual tonsil (magnification: 10,000x); and (right panel) trapped among the cilia of the nasopharyngeal tonsil (magnification: 7,000x). *[Source: Timoney, J. F., Kumar, P. & Muthupalani, S. (2006) Interaction of Streptococcus equi with the equine nasopharynx.* International Congress Series. *1289:267–270.]*

TABLE 13-1 Examples of Extracellular Bacteria and the Diseases They Cause

Pathogen	Disease
Bacillus anthracis	Anthrax
Borrelia burgdorferi	Lyme disease
Clostridium botulinum	Botulism
Clostridium tetani	Tetanus
Corynebacterium diphtheriae	Diphtheria
Escherichia coli O157:H7	Hemorrhagic colitis
Helicobacter pylori	Ulcers
Haemophilus influenzae	Bacterial meningitis
Neisseria meningitides	Bacterial meningitis
Neisseria gonorrhoeae	Gonorrhea
Staphylococcus aureus	Food poisoning, toxic shock
Streptococcus pyogenes	Strep throat, flesh-eating disease
Streptococcus pneumoniae	Pneumonia, otitis media
Treponema pallidum	Syphilis
Vibrio cholerae	Cholera
Yersinia enterocolitica	Severe diarrhea
Yersinia pestis	Bubonic plague

secreted but rather are released only when the cell walls of Gram-negative bacteria are damaged. A given Gram-negative bacterial species may supply both exotoxins and endotoxins.

Different exotoxins and endotoxins cause disease by different means and in different locations. For example, infection with *Vibrio cholerae* results in the local release of an exotoxin that binds to gut epithelial cells and induces the severe diarrhea that characterizes cholera. *Clostridium botulinum* produces a neuro-exotoxin that blocks the transmission of nerve impulses to the muscles, resulting in the paralysis characteristic of botulism. In contrast, damage to a host caused by an endotoxin is always immunopathic. The LPS of Gram-negative bacteria activates macrophages and induces them to release pro-inflammatory cytokines, particularly TNF and IL-1. As described in Box 3-2 in Chapter 3, although a little TNF and IL-1 is a good thing, the very high concentrations of these cytokines that are secreted in response to a significant Gram-negative bacterial infection can induce high fever and endotoxic (septic) shock.

NOTE: The ability of an individual to fight off infection can be influenced by the particular allele of a given PRR gene he/she expresses. As defined in Chapter 6, the varying nucleotide sequences of alleles of the same gene are known as *polymorphisms*. **Single nucleotide polymorphisms (SNPs)** are alleles that differ from the cognate gene by one nucleotide. It is estimated that there are ~10 million SNPs in the human genome. An SNP may affect the rate of transcription or translation of the resulting protein product, its amino acid sequence, its stability and half-life, its interaction with receptors, and/or its function. For example, a particular TLR4 SNP is associated with an increased risk of endotoxic shock following infection by Gram-negative bacteria, while a certain TLR2 SNP renders individuals highly susceptible to endotoxic shock following infection by Gram-positive bacteria. A database of defined human SNPs is maintained by the U.S. National Institutes of Health at www.ncbi.nlm.nih.gov/projects/SNP so that medical scientists can easily access this growing resource.

II. Immune Effector Mechanisms

i) Humoral Defense

Because extracellular bacteria cannot routinely "hide" within host cells, antibodies are generally highly effective against these species. Polysaccharides present in bacterial cell walls make perfect Ti antigens for B cell activation (**Fig. 13-1, #1**), while other bacterial components supplying Td antigens induce primarily a Th2 response that provides T help for antibacterial B cells (**#2**). Neutralizing IgM antibodies dominate in the vascular system, while smaller IgG antibodies protect the tissues. These antibodies neutralize bacteria by physically preventing them from attaching to host cell surfaces (**#3**). Even though they do not need to enter host cells for replication, most extracellular bacteria try to adhere to host cells to avoid being swept off or out of the host by skin sloughing or movement of the intestinal contents. Antibodies can also serve as opsonins, coating the bacterium such that it is engulfed by phagocytic leukocytes expressing FcRs (**#4**). Once captured inside the phagocyte, extracellular bacteria are usually very vulnerable to killing via pH changes, defensins, and the ROIs and RNIs associated with the phagosomal respiratory burst. Antibodies made against bacterial exotoxins are called **antitoxins**. Antitoxins neutralize a toxin by preventing it from binding to the cells it would otherwise damage (**#5**). 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 or diphtheria relies solely on antitoxins directed against the *Clostridium tetani* exotoxin or *Corynebacterium diphtheriae* exotoxin, respectively.

ii) Complement

All three pathways of complement activation can be brought to bear on extracellular bacteria (**Fig. 13-1, #6**). Antibacterial antibodies of the appropriate isotype (particularly IgM) will bind to complement component C1q to trigger the classical cascade. The alternative pathway can be activated by the binding of C3b to peptidoglycan in Gram-positive bacterial cell walls or LPS in Gram-negative bacterial cell walls. The lectin pathway is activated by the binding of MBL to distinctive sugars arrayed on bacterial cell surfaces. Almost all types of extracellular bacteria can be eliminated by phagocytosis facilitated by the binding of opsonins such as C3b that are produced during complement activation. In addition, bacteria possessing a membrane can be dispatched by MAC-mediated lysis. Complement is particularly crucial for defense against the *Neisseria* group of Gram-negative bacteria.

Mechanisms of complement activation were illustrated in detail in Figure 3-7.

NOTE: There is growing evidence that Th17 responses linked to the apoptosis of infected host cells are important for defense against extracellular bacteria. Infection of a host with a pathogen that preferentially colonizes the mucosae, such as *S. pneumoniae* or *Helicobacter pylori*, often results in the generation of Th17 effector cells that play a key role in bacterial clearance. Some immunologists believe that an infected mucosal cell which undergoes apoptosis in an inflammatory environment furnishes a combination of PAMPs and DAMPs that induce DCs to produce TGFβ and IL-6. Any Th0 cell interacting with such a DC is then directed to undergo Th17 cell differentiation. In contrast, a host cell that undergoes routine apoptosis in the absence of infection produces only DAMPs that induce the DC to secrete TGFβ alone, a situation that favors iTreg cell generation (refer to Ch. 10).

III. Evasion Strategies

Strategies used by extracellular bacteria to evade immune responses are summarized in **Table 13-2**.

i) Interfere with Host PRRs

Some extracellular bacteria are able to manipulate the host's induced innate response by avoiding or modifying the outcome of PRR engagement. For example, *H. pylori* contains modified forms of LPS and flagellin that bind abnormally to TLR4 or TLR5, respectively, and fail to induce proper TLR signaling. *Y. enterocolitica* produces

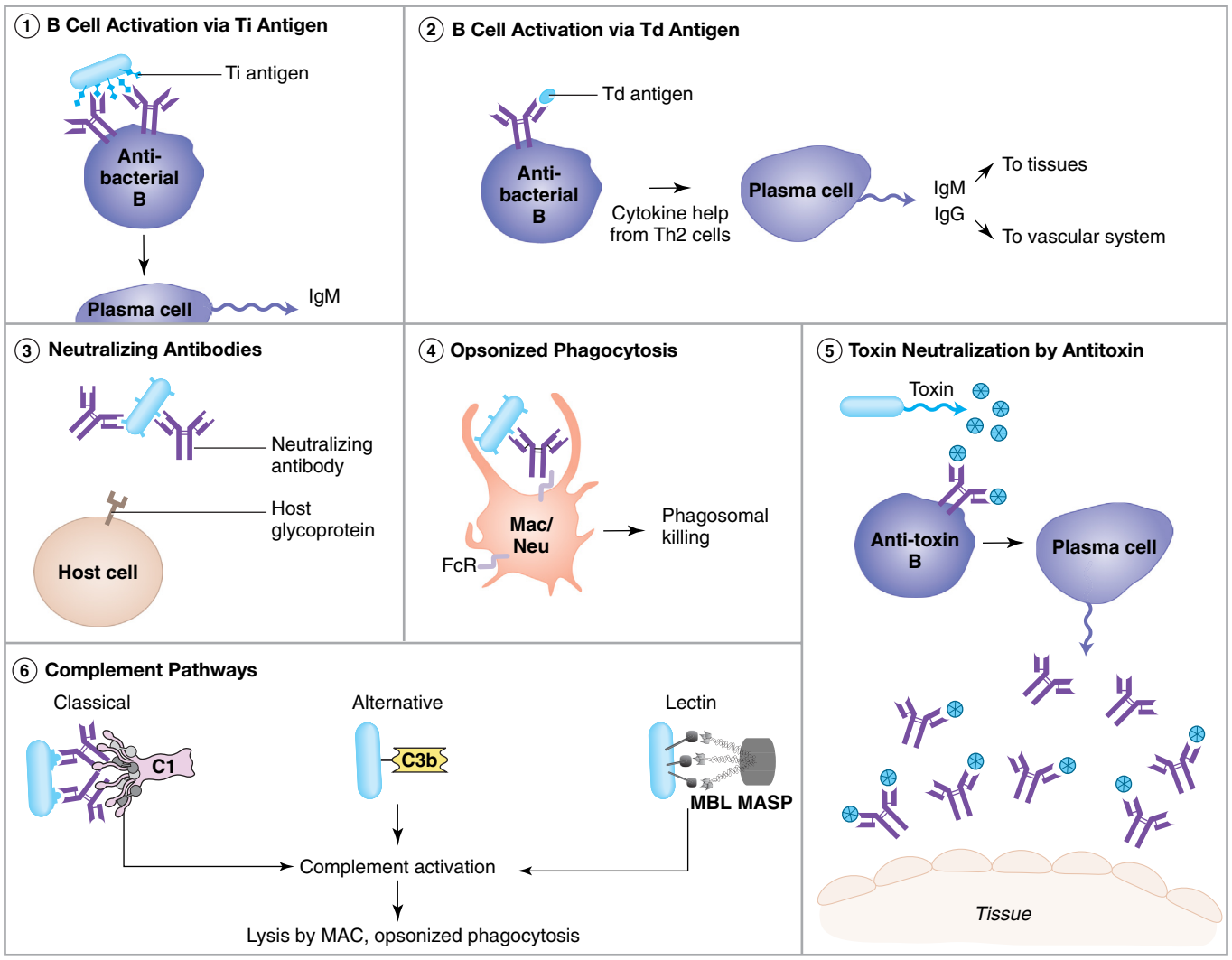

Fig. 13-1
Major Mechanisms of Immune Defense against Extracellular Bacteria

(**1**) Bacterial polysaccharides acting as Ti antigens activate B cells that generate plasma cells producing antibacterial IgM antibodies. (**2**) Bacterial Td antigens activate additional antibacterial B cells during Th2 responses. (**3**) Neutralizing antibodies recognizing bacterial components block bacterial access to host cell glycoprotein receptors. (**4**) Antibody-bound bacteria are recognized by FcRs on macrophages and neutrophils, which engulf and kill the pathogen. (**5**) Neutralizing antitoxin antibodies bind to bacterial toxin molecules and prevent them from damaging cell surfaces. (**6**) Bacteria bound by antibody plus C1, or C3b, or MBL activate complement.

a protein called the V antigen that binds to TLR2 and stimulates production of the immunosuppressive cytokine IL-10. This IL-10 then inhibits host cell secretion of IFNγ and TNF. Mice lacking TLR2 are thus actually less susceptible than wild type animals to *Y. enterocolitica* infection because their immune systems cannot be co-opted in this way and continue to produce IFNγ and TNF.

ii) Avoid Antibodies

Some extracellular bacteria, such as the *Gonococci*, ensure their adhesion to host tissues by routinely and spontaneously changing the amino acid sequence of the bacterial proteins used to stick to the host cell surface. Neutralizing antibodies directed against the original bacterial protein may not "see" the new version, allowing the bacteria to establish an infection. Other bacteria secrete proteases that cleave antibody proteins and render them non-functional. For example, *Haemophilus influenzae* expresses IgA-specific proteases that degrade sIgA in the blood and SIgA in the mucus.

TABLE 13-2 Evasion of the Immune System by Extracellular Bacteria

Immune System Element Thwarted	Bacterial Mechanism
Host PRRs	Produce modified PAMPs Alter PRR signaling and produce IL-10
Antibodies	Alter expression of surface molecules Secrete anti-Ig proteases
Neutrophil recruitment	Secrete a toxin that blocks host cell production of neutrophil chemokines
Phagocytosis	Block binding of phagocyte receptors to bacterial capsule Hide temporarily in non-phagocytes Inject bacterial protein that disrupts phagocyte function
Complement	Prevent C3b binding by lack of suitable surface protein, steric hindrance by surface proteins, C3b degradation Inactivate various steps of complement cascade Capture host RCA proteins Induce host production of antibody isotypes that are poor complement-fixers

iii) Avoid Neutrophils

As we saw in Chapter 3, the chemotaxis and extravasation of neutrophils into a site of pathogen attack are among the first elements of induced innate defense. Studies of *Streptococcus pyogenes* have shown that this bacterium produces a toxin that blocks the production by host cells of the chemokines needed to draw neutrophils into an infected site. While *S. pyogenes* infection of the topmost layer of a tissue causes relatively mild disease (like strep throat), deeper infections can cause *necrotizing fasciitis* (flesh-eating disease), which can be lethal. Histological examination has revealed that this lethality correlates with a deficit in neutrophils in the affected tissue.

iv) Avoid Phagocytosis

The polysaccharide coating of encapsulated bacteria protects them from phagocytosis by conferring a charge on the bacterial surface that inhibits binding to phagocyte receptors. In addition, although C3b may still attach to the bacterial surface, the capsule sterically interferes with the binding of phagocyte receptors to the C3b so that opsonized phagocytosis of the bacterium is much less efficient. Some non-encapsulated extracellular bacteria avoid capture by phagocytes by temporarily entering non-phagocytes such as epithelial cells and fibroblasts. To gain access to these cells, the pathogens may inject bacterial proteins into the host cell that promote either macropinocytosis or cytoskeletal rearrangements facilitating bacterial uptake. Extracellular bacteria may also inject bacterial proteins that have direct antiphagocyte activity. For example, *Y. enterocolitica* injects into macrophages a bacterial phosphatase that binds to certain tyrosine-phosphorylated host proteins required for intracellular signaling and actin reorganization. When the bacterial phosphatase dephosphorylates these host proteins, phagocytosis of the bacterium is blocked.

v) Avoid Complement

Some extracellular bacteria can avoid complement by virtue of their basic structure. For example, *Treponema pallidum*, the organism that causes syphilis, has an outer membrane devoid of transmembrane proteins and so offers almost no place suitable for C3b deposition. Other bacteria have cell wall lipopolysaccharides that contain long, outwardly projecting chains that prevent the MAC from assembling on the bacterial surface. Many extracellular bacteria synthesize substances that inactivate various steps of the complement cascade. For example, group B *Streptococci* contain sialic acid in their cell walls that degrades C3b and blocks alternative complement activation. Other *Streptococci* produce proteins that bind to the normally 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. Certain *Salmonella*

RCA proteins are "regulators of complement activation" that are expressed on host cell surfaces and protect them from complement-mediated destruction (refer to Ch. 3).

species express proteins that interfere with the terminal steps of complement activation, while *Gonococci* and *Meningococci* induce the host to preferentially produce antibody isotypes (such as IgA) that are poor at fixing complement. These "blocking antibodies" compete with complement-fixing antibodies for binding to the bacterial surface, reducing MAC formation. Steric hindrance by blocking antibodies also interferes with C3b deposition.

C. Immunity to Intracellular Bacteria

I. Disease Mechanisms

A *vector* is an intermediary organism that introduces the pathogen into the ultimate host.

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. Once inside the host, intracellular bacteria elude phagocytes, complement and antibodies by moving right inside host cells to reproduce. Epithelial and endothelial cells, hepatocytes and macrophages are popular targets. Because macrophages are mobile, bacteria that infect these cells are quickly disseminated all over the body.

Intracellular bacteria generally enter host cells by clathrin-mediated endocytosis and are thus first confined to a clathrin-coated vesicle. Some species remain in the vesicle, whereas others escape and take up residence in the cytoplasm. 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 cell and do not produce tissue-damaging bacterial toxins. However, their intracellular lifestyle makes these organisms difficult to eradicate completely and chronic disease may result. Examples of diseases caused by intracellular bacteria appear in **Table 13-3**.

II. Immune Effector Mechanisms

i) Neutrophils and Macrophages

Early infections by intracellular bacteria are frequently controlled by the defensins secreted by neutrophils because these proteins can destroy the invaders before they can take refuge inside a host cell (**Fig. 13-2, #1**). Those bacteria that escape the defensins and are taken up by neutrophil phagocytosis find themselves, not in a haven for replication, but rather within a phagosome that can kill them via the powerful respiratory burst. Memory Th17 cells have an important role to play here, as the IL-17 they secrete recruits neutrophils to the site of invasion and promotes phagocytosis. Memory Th17 cells also

TABLE 13-3 Examples of Intracellular Bacteria and the Diseases They Cause

Pathogen	Disease
Bordetella pertussis	Diphtheria (whooping cough)
Brucella melitensis	High fevers, brucellosis
Chlamydia trachomatis	Eye and genital diseases
Legionella pneumophila	Legionnaire's disease
Listeria monocytogenes	Listeriosis
Mycobacterium leprae	Leprosy
Mycobacterium tuberculosis	Tuberculosis
Mycoplasma pneumoniae	Atypical pneumonia
Rickettsia rickettsii	Rocky Mountain spotted fever
Salmonella typhi	Typhoid fever
Salmonella typhimurium	Food poisoning
Shigella flexneri	Enteric disease

recruit activated macrophages and stimulate both their phagocytic activity and production of the IL-12 needed for Th1 cell differentiation (**#2**). For both neutrophils and macrophages, the killing of phagocytosed bacteria is frequently enhanced by certain host proteins present within the phagolysosomal membrane, and also by host enzymes in the ER or Golgi that regulate the maturation of pathogen-containing phagosomes. These enzymes are greatly upregulated in response to IFNs or LPS. In addition to phagocytosis, macrophages activated by TLR engagement produce pro-inflammatory cytokines that promote NK cell activation and Th1 differentiation (see following sections).

NOTE: The role of TLRs in defense against intracellular bacteria has been highlighted by the fact that lipoprotein and lipoglycan components of *Mycobacteria* are readily recognized by TLR2 and TLR4. In addition, from a clinical perspective, certain SNPs in TLR5 render individuals highly susceptible to Legionnaire's disease, a form of pneumonia caused by the flagellin-expressing intracellular bacterium *L. pneumophila*.

ii) NK Cells and γδ T Cells

NK cells stimulated by macrophage-derived IL-12 detect infected host cells by their deficit in MHC class I expression (which is typically downregulated by the infection) and destroy them by natural cytotoxicity (**Fig. 13-2, #3**). In addition, activated NK cells secrete copious amounts of IFNγ, which promotes macrophage activation directly and Th1 cell differentiation indirectly. γδ T cells are also important in combatting at least some intracellular infections. Many species of intracellular bacteria (particularly the *Mycobacteria*) release small phosphorylated molecules as they attempt to colonize the host. These metabolites trigger the generation of γδ T cell effectors that either carry out cytolysis or secrete IFNγ (**#4**).

iii) CD8⁺ T Cells

CTLs are critical for resolving many intracellular bacterial infections. If the bacterium replicates in the cytosol of the infected cell, some of its component proteins enter the endogenous antigen processing pathway and are presented on MHC class I, marking the cell as a target for CTL-mediated destruction (**Fig. 13-2, #5**). These CTLs are generated from pathogen-specific naïve Tc cells that were activated in the draining lymph node. This Tc activation is initiated by DCs that acquired antigens derived from the degradation of a phagocytosed bacterium or a dying host cell, followed by cross-presentation of peptides from these antigens on MHC class I. Interestingly, CTLs rarely use Fas-mediated apoptosis or perforin/granzyme-mediated cytolysis to kill target cells infected with intracellular bacteria, in contrast to their destruction of virus-infected cells (see below). Rather, CTLs eliminate these targets by relying on secreted TNF and IFNγ and/or granule components with direct antimicrobial activity. Accordingly, individuals lacking the IFNγ receptor are highly susceptible to *Mycobacterial* infections.

iv) CD4⁺ T Cells

$CD4^+$ T cells make a significant contribution to defense against intracellular bacteria (see **Box 13-1**), not only because of the IL-2 they secrete to support Tc differentiation but also because Th1 cells are required for macrophage hyperactivation. It is not unusual for intracellular bacteria phagocytosed by macrophages to be resistant to routine phagosomal killing, and the IFNγ produced by activated Th1 effectors hyperactivates the macrophages such that they gain enhanced microbicidal powers. The sequence of events starts when bacterial antigens either secreted by the bacteria themselves or released by necrotic infected cells are taken up by DCs. In the local lymph node, peptides from these antigens are bound to MHC class II and presented to $CD4^+$ T cells (**Fig. 13-2, #6**). IL-12 produced by macrophages favors the differentiation of Th1 effectors, which supply the intercellular contacts (particularly CD40L) and IFNγ that drive macrophage hyperactivation. A hyperactivated macrophage produces large quantities of ROIs and RNIs that efficiently kill almost all intracellular pathogens. If the bacterium is still resistant, however, a hyperactivated macrophage may go on to participate in formation of a **granuloma** (see later) in order to contain the threat.

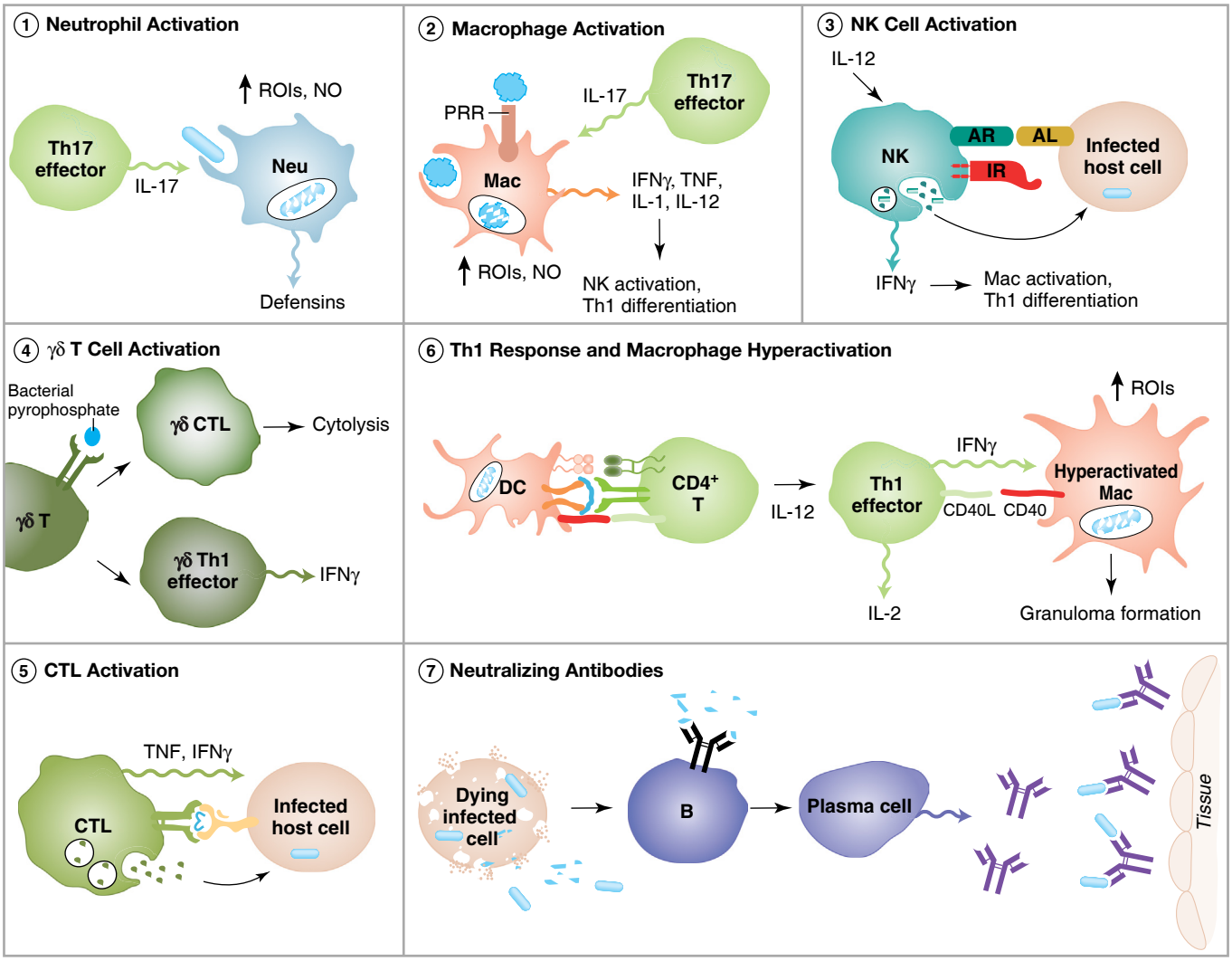

Fig. 13-2
Major Mechanisms of Immune Defense against Intracellular Bacteria

(**1**) Th17 cell-derived IL-17 recruits neutrophils to a site of infection where they capture intracellular bacteria by phagocytosis, kill them via the respiratory burst, and produce antimicrobial peptides. (**2**) IL-17 also recruits macrophages that are then activated by TLR engagement or phagocytosis of intracellular bacteria. These cells initiate phagosomal killing and secrete pro-inflammatory cytokines. (**3**) NK cells activated by IL-12 kill infected host cells by natural cytotoxicity and secrete IFNγ. (**4**) Bacterial phosphorylated metabolites activate γδT cells. (**5**) CTLs recognizing bacterial peptides presented by an infected host cell kill it by releasing toxic granule contents and/or cytokines. (**6**) Infected DCs present bacterial peptides on MHC class II to $CD4^+$ T cells, which generate Th1 effectors in the presence of IL-12. These Th1 cells supply cytokines for the CTL response and macrophage hyperactivation. (**7**) Bacterial components released from a dying infected cell activate B cells to produce neutralizing antibodies that intercept any bacterium that is temporarily extracellular.

v) Humoral Defense

Antibodies can make an important contribution to host defense against at least some intracellular bacteria. Bacterial components released from a dying infected cell may activate B cells to produce neutralizing antibodies (Fig. 13-2, #7). These antibodies may bind to newly arrived bacteria or to bacterial progeny that have been released into the extracellular milieu but have not yet infected a fresh host cell. The antibody-bound bacteria are unable to enter host cells and are eliminated by opsonized phagocytosis or classical complement-mediated lysis, curbing pathogen spread.

vi) Granuloma Formation

When an intracellular pathogen like *Mycobacterium tuberculosis* is able to resist killing by CTLs and hyperactivated macrophages, the body attempts to wall off the pathogen

Box 13-1 Lessons from Leprosy

The importance of the Th1 response to defense against intracellular pathogens is clearly illustrated in human immunity to *Mycobacterium leprae* infection. Individuals who are predisposed to mounting Th2 responses (i.e., their Th cells preferentially secrete IL-4 and IL-10) and are infected with *M. leprae* suffer from a devastating form of leprosy known as *lepromatous leprosy*. The DCs in the epidermis and dermis of these patients exhibit reduced expression of the costimulatory molecule B7, which further compromises the effectiveness of the T cell response. In contrast, individuals who usually mount Th1 responses (i.e., their Th cells preferentially secrete IFNγ) and are infected with *M. leprae* present with *tuberculoid leprosy*, which is generally a less severe form of the disease. The cell-mediated immunity favored by a Th1 response is clearly more effective against this intracellular pathogen than the Th2 response that promotes humoral immunity.

Recent studies have shown that TLRs are intimately involved in the balance between lepromatous and tuberculoid leprosy. DCs and monocytes in lesions of patients with tuberculoid leprosy show strong TLR2 and TLR1 expression, but DCs and monocytes in lesions of patients with lepromatous leprosy do not. A heterodimer of TLR1/TLR2 forms a PRR that recognizes a lipoprotein of *M. leprae*. Engagement of this TLR1/TLR2 complex normally results in leukocyte production of IL-2, IL-12, TNF and IFNγ, which influence nearby DCs to induce Th1 differentiation. In the absence of normal TLR1/TLR2 signaling, however, the leukocytes tend to produce IL-10, which induces DCs to promote Th2 differentiation. Thus, a lower level of the TLR1/TLR2 complex, or a failure in the TLR1/TLR2 signaling pathway, most often favors the development of the more severe form of leprosy.

in a cellular structure called a **granuloma** that forms around the infected macrophages (**Plate 13-2**). The inner layer of a granuloma contains macrophages and $CD4^+$ T cells, whereas the exterior layer is composed of $CD8^+$ T cells. Eventually, the granuloma exterior 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 is 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. If the granuloma breaks down, the trapped pathogens are released back into the body to resume replication. Should the host be immunosuppressed and unable to marshal the T cells and macrophages necessary to fight this fresh assault, the pathogen may reach the blood. As the bacteria travel in the circulation, they can infect organs throughout the body and even precipitate death.

Cytokines play a critical role in granuloma formation. IL-17 production by Th17 cells is required for Th1 effector recruitment and the stimulation of IL-12 production by macrophages. Sustained IFNγ production by Th1 cells and CTLs is needed to maintain macrophage hyperactivation. TNF production by hyperactivated macrophages is crucial not only for early chemokine synthesis (to recruit leukocytes to the incipient granuloma) but also for aggregating these cells and establishing the "wall" around the invaders. IL-4 and IL-10 secreted by Th2 cells late in an adaptive response control granuloma formation, damping it down as the bacterial threat is contained. Recent work has shown that TLR signaling also influences granuloma formation and thus the

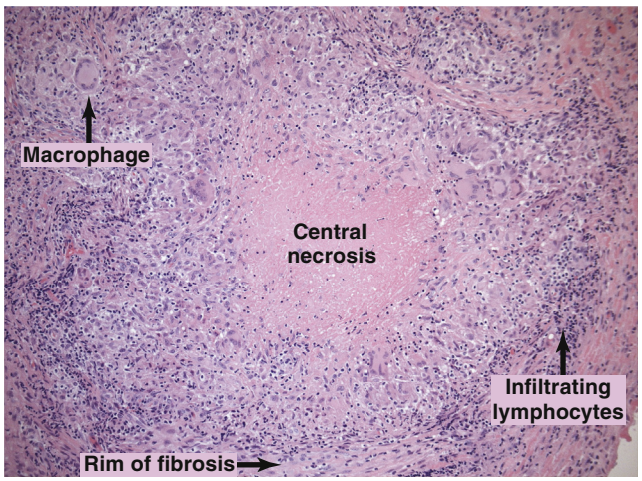

Plate 13-2
Granuloma Cross-section

A central zone of necrosis is surrounded by activated macrophages. The rim of fibrosis, infiltrating lymphocytes, and a macrophage are indicated. *[Reproduced by permission of David Hwang, Department of Pathology, University Health Network, Toronto General Hospital.]*

TABLE 13-4 Evasion of the Immune System by Intracellular Bacteria

Immune System Element Thwarted	Bacterial Mechanism
Host PRRs	Produce modified PAMPs that inhibit normal signaling Produce modified PAMPs that trigger abnormal signaling which inhibits APCs
Phagosomal destruction	Infect a non-phagocyte Synthesize molecules blocking lysosomal fusion, phagosomal acidification, ROI/RNI killing Recruit host proteins blocking lysosome function
Hyperactivated macrophages	Block expression of host genes needed for macrophage hyperactivation
Antibodies	Spread to new host cell via pseudopod invasion
T cells	Reduce antigen presentation by APCs Induce DCs to produce immunosuppressive cytokines

outcome of infections by pathogens normally contained by them. For example, certain SNPs in TLR9 and NOD2 appear to increase susceptibility to *M. tuberculosis* and the TB it causes, while some TLR8 SNPs are linked to TB resistance.

III. Evasion Strategies

Evasion strategies used by intracellular bacteria are summarized in **Table 13-4**.

i) Interfere with Host PRRs

Like certain extracellular bacteria, some intracellular bacteria, including *L. pneumophila*, produce modified forms of TLR ligands such as LPS. These ligands inhibit PRR signaling and block the activation of innate leukocytes. Operating in the opposite way, *M. tuberculosis* produces a small lipoprotein that binds fiercely to host TLR2 and prolongs its signaling. This abnormal signaling inhibits IFNγ production and antigen processing by APCs, downregulating T cell responses and allowing the bacteria to persist.

ii) Avoid Phagosomal Destruction

Some intracellular bacteria avoid phagosomal killing by replicating in non-phagocytic cells. For example, *M. leprae* infects the Schwann cells of the human peripheral nervous system. Other intracellular bacteria deliberately enter phagocytes but then inactivate them or take steps to escape phagosomal killing. For example, *L. monocytogenes* accesses mouse phagocytes via host FcRs and CRs but then synthesizes a protein called *listeriolysin O* (LLO) that induces pore formation in the phagolysosomal membrane. The bacterium escapes through the pore into the relative safety of the cytoplasm. *B. pertussis* expresses a surface receptor that binds to a glycoprotein found primarily on phagocytes, promoting deliberate engulfment of the bacterium. Once inside the phagocyte, *B. pertussis* neutralizes the respiratory burst and inhibits other bactericidal activities, allowing the pathogen to persist within the host cell. When *M. tuberculosis* finds itself being engulfed in a macrophage phagosome, it recruits to the phagosome a host protein called TACO that inhibits the fusion of the phagosome to lysosomes. *M. tuberculosis* also produces NH_4^+, which reverses the acidification of phagolysosomes and promotes fusion with harmless endosomes. In addition, *M. tuberculosis* infection interferes with the expression of host genes needed for microbicidal action and macrophage hyperactivation. As a result of all these measures, *Mycobacteria* can survive within host phagosomes for long periods. Certain *Salmonella* species produce molecules that decrease the recruitment of NADPH oxidase to the phagolysosome, inhibiting ROI and RNI generation. Other intracellular bacteria block phagosomal ROIs and RNIs either by chemically neutralizing them or by synthesizing the enzymes superoxide dismutase and catalase that break down ROIs, RNIs and hydrogen peroxide.

iii) Avoid Antibodies

Some intracellular pathogens avoid the humoral response by moving directly from one host cell to another, giving antibodies no chance to bind. For example, in mice, *L. monocytogenes*

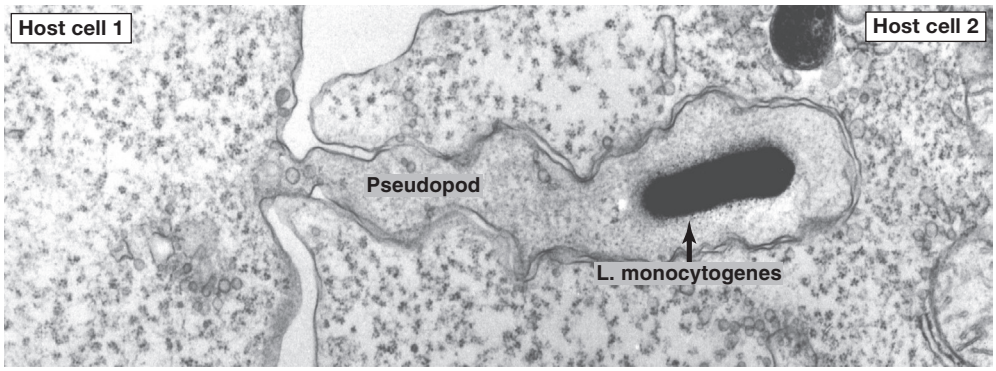

Plate 13-3
Pseudopod Invasion

A pseudopod containing a *Listeria monocytogenes* bacterium is extended by an infected cell (Host cell 1) and engulfed by an uninfected neighboring cell (Host cell 2), allowing the bacterium to spread without exposure to host antibody. *[Reproduced by permission.]*

can induce the actin-based formation of a pseudopod that invaginates into a neighboring non-phagocytic cell (**Plate 13-3**). The neighboring cell engulfs the bacterium-containing pseudopod and confines it in a vacuole. The bacterium then uses LLO and phospholipases to break out of the vacuole and enter the cytoplasm of the new cell. Because the bacterium is never exposed in the extracellular milieu, it never becomes an antibody target.

iv) Avoid T Cells

Some intracellular bacteria avoid stimulating T cell responses by interfering with APC function. For example, infection of DCs by *M. tuberculosis* promotes downregulation of the expression of MHC class I, MHC class II and CD1. Antigen presentation to T cells and NKT cells is thus inhibited. *B. pertussis* alters the functions of DCs by inducing them to switch to IL-10 production, thereby suppressing the antipathogen response.

D. Immunity to Viruses

I. Disease Mechanisms

Viruses are stripped-down intracellular pathogens that consist of a nucleic acid genome packaged in a protein coat called a *capsid*. The viral genome may be DNA or RNA, and the capsid may or may not be covered in a membranous structure called an *envelope*. Most viruses enter a host cell by binding to a host surface receptor. Replication of the viral genome and synthesis of viral mRNAs follow, which may be carried out by host or viral enzymes, depending on the virus. However, all viruses lack protein synthesis machinery and rely on the host cell for viral protein translation and progeny virion assembly. Progeny virions released from an infected cell attack neighboring host cells and initiate new replicative cycles that lead to widespread dissemination of the virus. Progeny virions that reach the blood are free to spread systemically. Examples of diseases caused by viruses are given in **Table 13-5**.

Viruses cause disease both directly and indirectly. Viruses frequently kill or at least inactivate host cells, depriving the host of these cells' normal functions such that clinical symptoms appear. As well, the immune response to the viral infection frequently damages host tissues and induces inflammation, causing immunopathic disease. Clinicians classify diseases caused by viruses as either *acute* or *chronic*. When a host is initially infected with a virus, the host experiences **acute disease** in that the illness may be mild or severe (depending on the degree of pathogenicity or **virulence** of the virus) but is only short term in duration. An effective immune response removes the virus completely from the body. However, sometimes viruses are not completely eliminated during the acute infection and remain in the body to establish **persistent infections**. The ongoing low levels of viral replication associated with these persistent infections cause long-term or recurrent illnesses that are considered **chronic diseases**. In some cases, a host will experience no chronic disease symptoms at all if his/her cell-mediated immune response is effective enough to block the assembly of new virus particles. The spread of the virus to fresh host cells is halted, and the virus then persists in the body in an inactive state and does not

TABLE 13-5 Examples of Viruses and the Diseases They Cause

Pathogen	Disease
Adenovirus	Acute respiratory infections
Cytomegalovirus (CMV)	Pneumonitis, hepatitis
Ebola virus	Hemorrhagic fever
Epstein–Barr virus (EBV)	Infectious mononucleosis, Burkitt's lymphoma
Hepatitis viruses (HVA, HVB, HVC)	Hepatitis, cirrhosis, liver cancer
Herpes simplex (HSV)	Cold sores
Human immunodeficiency virus (HIV)	Acquired immunodeficiency syndrome (AIDS)
Human papilloma virus (HPV)	Skin warts, genital warts, cervical cancer
Human T cell leukemia virus 1 (HTLV-1)	T cell leukemias and lymphomas
Influenza virus	The "flu"
Kaposi's sarcoma herpes virus (KSHV)	Kaposi's sarcoma
Measles virus (MV)	Measles
Poliovirus	Poliomyelitis, post-polio fatigue
Polyoma virus	Infections of respiratory system, kidney, brain
Rabies virus	Rabies
Rhinovirus	Common cold
SARS (severe acute respiratory syndrome) virus	Severe acute respiratory syndrome
Vaccinia virus	Asymptomatic in most healthy humans, or mild rash and fever
Varicella zoster virus (VZV)	Chicken pox, shingles
Variola virus	Smallpox
West Nile virus (WNV)	Flu-like illness, fatigue, encephalitis

replicate. However, if the host's cell-mediated response weakens due to aging or immunosuppression, the latent virus reactivates, replicates and again causes acute disease. For example, the reactivation of latent varicella zoster virus (VZV), which causes chicken pox in young children, precipitates the painful adult skin condition known as shingles.

II. Immune Effector Mechanisms

i) Interferons and the Antiviral State

Production of the multifunctional cytokines IFNα, IFNβ and IFNγ is one of the earliest innate responses induced by viral infections. IFNα and IFNβ are secreted primarily by host cells infected with a virus, whereas IFNγ is initially produced by activated macrophages and NK cells and later on by activated Th1 cells. Any one of these IFNs can initiate a series of metabolic and enzymatic events in an uninfected host cell that results in it adopting an **antiviral state** (**Fig. 13-3, #1**). A host cell in the antiviral state can take enzymatic action to prevent an attacking virus from invading or starting to replicate. Both the transcription and translation of viral mRNAs and proteins are inhibited.

Another key source of IFNα and IFNβ is the plasmacytoid DC population described in Chapter 7. These cells are specialized in the use of endosomal PRRs, particularly TLR9, to sense viral RNA and DNA. Activated pDCs then respond rapidly with vigorous production of IFNα and IFNβ. For example, the herpes simplex viruses HSV1 and HSV2 have DNA genomes that are unusually rich in the CpG motif, which is a ligand of TLR9. Accordingly, pDCs are vital for immune defense against these viruses.

ii) NK Cells

Although CTLs are the prime mediators of the cell-mediated immunity needed to eliminate viruses (see later), there is often a 4–6-day delay before these cells can expand to sufficient numbers to complete the task. Where a virus causes downregulation of

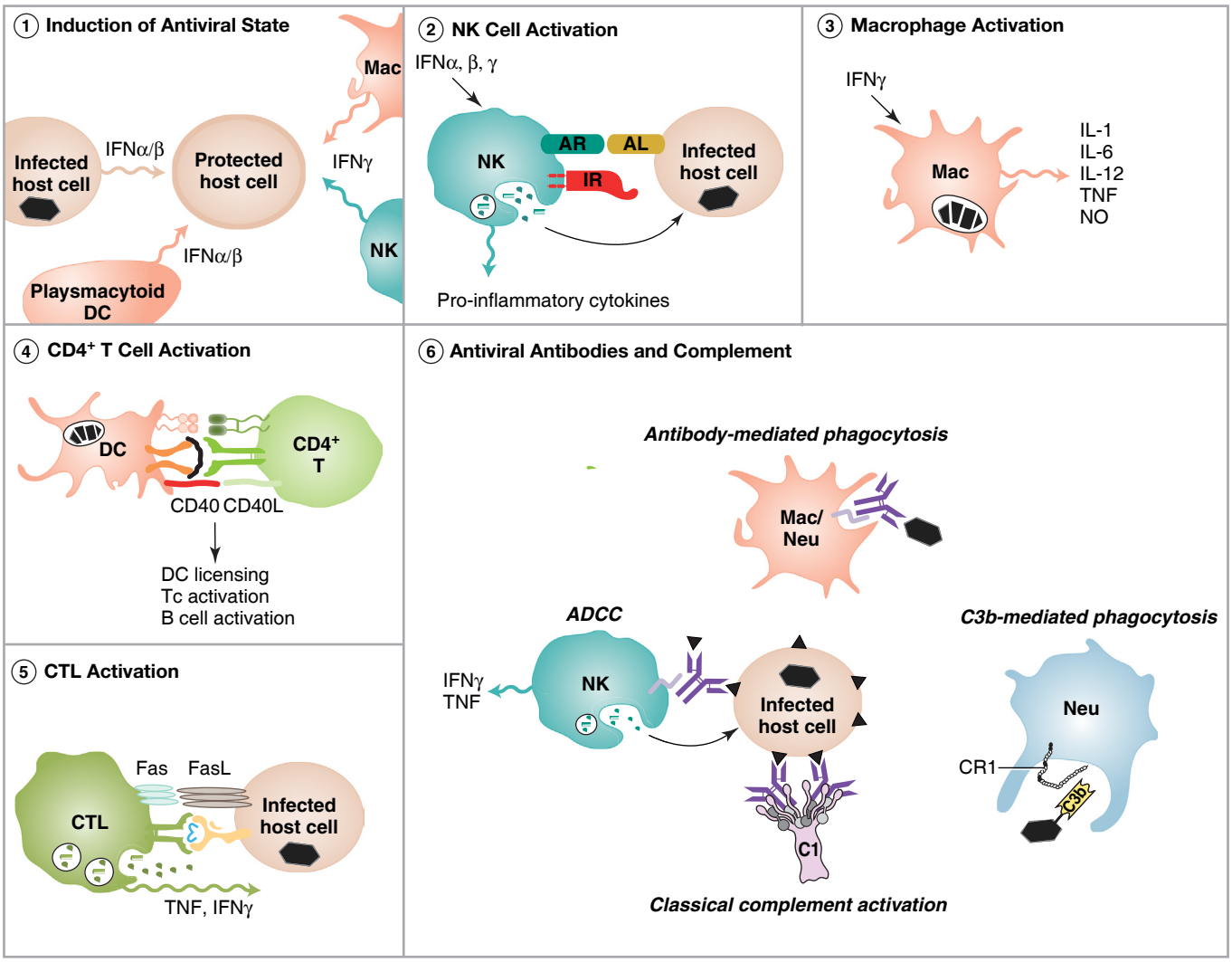

Fig. 13-3
Major Mechanisms of Immune Defense against Viruses

(1) IFNα/β secreted by infected host cells and pDCs, and IFNγ secreted by activated macrophages and NK cells, cause uninfected host cells to adopt an antiviral state. **(2)** Activated NK cells secrete cytokines and kill infected host cells that fail to express sufficient peptide-MHC class I. **(3)** Activated macrophages efficiently capture and kill viruses, and produce NO and cytotoxic cytokines. **(4)** Infected DCs, or those that have captured virions or viral products, activate CD4+ T cells, which reciprocally license DCs for Tc cell activation. **(5)** Antiviral CTLs kill virus-infected host cells by Fas killing, cytotoxic cytokines, or perforin/granzyme-mediated cytotoxicity. **(6)** Antibodies bound to a viral antigen on the surface of an infected host cell may engage FcRs on an NK cell, macrophage, or neutrophil and trigger ADCC or opsonized phagocytosis. If the bound antibody binds to C1, classical complement activation can lead to MAC-mediated destruction of the infected cell. Virus particles bound to free C3b may bind to CR1 and be taken up by opsonized phagocytosis.

MHC class I on the host cell surface, direct cytolysis of infected cells by NK cells (via natural cytotoxicity) and NK production of inflammatory cytokines can supply early defense (Fig. 13-2, #2). Indeed, individuals whose NK cells are not fully functional show increased susceptibility to virus infection, especially by herpesviruses. Natural cytotoxicity and inflammatory cytokine production by NK cells are stimulated by all three IFNs. NK cells are also important mediators of antiviral ADCC. The upregulation of FcR expression on both NK cells and macrophages is stimulated by IFNγ.

iii) Macrophages

Whole virions or their components may be taken into a macrophage by clathrin-mediated endocytosis or phagocytosis. Numerous viral components, including viral DNA,

Most DCs express the same array of TLRs as macrophages and are also activated by viral PAMPs.

dsRNA, ssRNA, envelope proteins and surface glycoproteins, serve as PAMPs that can bind to macrophage PRRs such as TLR2, TLR3, TLR4, TLR7, TLR8 and TLR9. Indeed, a TLR2 SNP that abrogates TLR signaling increases susceptibility to cytomegalovirus (CMV) infection. Macrophages activated by PRR engagement during the course of a virus infection produce copious amounts of pro-inflammatory cytokines such as IL-12 and TNF (**Fig. 13-3, #3**). The presence of IFNγ in the milieu greatly enhances this function and also allows the macrophage to express the iNOS enzyme that generates NO. This NO facilitates macrophage production of ROIs and RNIs that will aid in killing phagocytosed viruses. Macrophages can also eliminate viruses via ADCC.

iv) CD4+ T Cells

TLR-stimulated DCs readily process viral proteins via the exogenous pathway and display viral peptides on MHC class II to activate naïve $CD4^+$ T cells (**Fig. 13-3, #4**). Th cells are important for defense against most viruses because these cells both license DCs and supply IL-2 for naïve $CD8^+$ Tc cell activation. Interaction of DCs with Th effector cells reciprocally spurs the production by the DC of pro-inflammatory mediators that recruit additional innate and adaptive leukocytes. Th cells also provide the CD40L-mediated costimulation and cytokines required for B cells to mount antibody responses to viral Td antigens.

v) CD8+ T Cells

CTLs are crucial for immune defense against most viruses. Because these pathogens replicate intracellularly, viral antigens are displayed on MHC class I on infected host cell surfaces and mark these cells as CTL targets. The effector CTLs generated from Tc cells activated in the draining lymph node return to the site of infection and kill the virus-infected cells via perforin/granzyme-mediated cytotoxicity, Fas-mediated apoptosis, or TNF and/or IFNγ secretion (**Fig. 13-3, #5**).

NOTE: Recent studies have identified small populations of $CD4^+$ (as opposed to $CD8^+$) cytotoxic T cells as being important for fighting persistent virus infections. In these cases, the naïve $CD4^+$ cells within the infected host gradually lose the ability to generate Th effectors and instead generate progeny that acquire cytotoxic properties, allowing them to kill infected host cells via perforin/granzyme-mediated cytotoxicity or Fas killing. These cells are the subject of much ongoing research.

vi) Humoral Defense

Because a virus is an intracellular pathogen, it is often out of the reach of antibodies during the primary adaptive response. Nevertheless, naïve B cells may recognize viral components displayed on the surface of an infected host cell or may encounter progeny virions as they are released from an infected cell. With the appropriate T cell help, these B cells are activated and generate plasma cells and memory B cells that are usually vital for complete resolution of the infection. Late in the primary response, neutralizing antibodies are released into the circulation and block further spread of the virus. As well, in a subsequent attack, the virus will have a harder time infecting the host because the circulating neutralizing antibodies rapidly bind to the virus and bar its access to host cell receptors. Antiviral antibodies may also initiate classical complement activation. The formation of the MAC on the surface of an enveloped virus or an infected host cell kills it, and the complement components that are produced during the cascade may opsonize extracellular virions and promote their uptake by phagocytosis (**Fig. 13-3, #6**). The antiviral antibodies themselves may also serve as opsonins. Finally, antibodies that have recognized viral antigens on infected host cell surfaces may engage FcRs on phagocytes and other leukocytes (particularly NK cells) and provoke ADCC.

It should be noted that some viruses are combatted (at least in part) by B cell responses that do not require T cell help. Viruses such as vesicular stomatitis virus (VSV) have highly repetitive structures on their surfaces that induce a Ti response. Ti responses are typically faster than Td responses because a Ti response involves only a B cell and does not require

B–T cell cooperation. An antiviral Ti response can function early in an infection to minimize the spread of the virus until antibodies against viral Td antigens can be synthesized.

vii) Complement

As well as the classical complement activation that is part of the humoral response, surface components of virions can directly activate the lectin and alternative complement pathways. Opsonization of viruses by C3b (or C3d) promotes phagocytosis by neutrophils and macrophages (refer to **Fig. 13-1, #6**).

III. Evasion Strategies

Viruses with small genomes 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 latter pathogens have developed ways of interfering with various components of the host immune response that allow them sufficient time to establish an infection. Once infection has occurred, many viruses hide from the immune system. Others confront the immune response head on by interfering with host cell signaling pathways. Evasion strategies used by viruses are summarized in **Table 13-6**.

TABLE 13-6 Evasion of the Immune System by Viruses

Immune System Element Thwarted	Viral Mechanism
Detection	Become latent
Antibodies	Alter viral epitopes via antigenic drift or shift Express viral FcR that blocks ADCC, neutralization and/or complement activation Block B cell intracellular signaling or activation
$CD8^+$ T cells	Infect cells with very low MHC class I expression Block MHC class I-mediated antigen presentation, including via miRNA Force pMHC internalization
$CD4^+$ T cells	Avoid infection of DCs Interfere with MHC class II-mediated antigen presentation Force pMHC internalization
NK cells	Express viral homologs of MHC class I Increase host synthesis of HLA-E or classical MHC class I Block MICB expression via miRNA
DCs	Block DC development or maturation Block DC upregulation of costimulatory molecules Block DC expression of CCR7 Upregulate DC expression of FasL
Complement	Block convertase formation Express viral homologs of host RCA proteins Increase expression of host RCA proteins Bud through host membrane and acquire host RCA proteins
Host PRRs	Produce proteins interfering with normal PRR signaling Have a genome low in CpG motifs
Antiviral state	Block secretion of IFNs Interfere with metabolic/enzymatic events that establish the antiviral state
Apoptosis	Block various steps of extrinsic or intrinsic pathways Express homologs of death receptors and regulatory molecules Express molecules sustaining host cell survival
Cytokines/chemokines	Express competitive inhibitors of cytokines and chemokines Block cytokine/chemokine transcription Block cytokine/chemokine translation via miRNA Downregulate host cytokine/chemokine receptor expression

i) Latency

Some persistent viruses avoid removal by the immune system through **latency**. When a virus adopts a latent state, it persists in the host cell in a defective form that renders it non-infectious for a period of time. In most cases, latency involves the inactivation of viral gene transcription needed for productive infection and the subsequent expression of new viral transcripts required for latency. Reversal from latency back to productive infection requires some type of reactivation of the productive infection genes that can occur only when the host's immune system has weakened.

Different viruses achieve latency in different ways. HIV integrates a cDNA copy of its RNA genome into the DNA of its host cell in such a way that there is limited transcription of viral genes. The DNA genomes of VZV and HSV do not integrate into the host DNA but instead form a complex with host nucleosomal proteins that block transcription of productive infection genes. A similar latency mechanism operates in cases of Epstein–Barr virus (EBV) and Kaposi's sarcoma herpesvirus (KSHV) infection. However, the latency of these viruses is associated with the development of tumors in the host: B cell lymphomas and nasopharyngeal carcinomas in the case of EBV, and the AIDS-related Kaposi's sarcoma in the case of KSHV.

The structure and life cycle of HIV is described further in Chapter 15.

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 an individual's existing memory lymphocytes or antibodies. This mechanism is most effective in long-lived hosts (like humans) that can sustain multiple re-infections, and is particularly important if the virus lacks the ability to become latent. The rapid modification of viral antigens through random mutations is known as **antigenic drift**. For example, like all RNA viruses, influenza virus cannot proofread its RNA genome during replication and thus sustains a high rate of mutation. The hemagglutinin (H) and neuraminidase (N) proteins, which are the only two viral proteins present on the surface of the influenza virion, are thus subtly different from viral generation to generation. These minor virus variants often replicate preferentially in the host, because they are not neutralized by antibodies raised against earlier strains. New influenza strains created by antigenic drift are responsible for localized influenza outbreaks. HIV is another virus that undergoes very rapid antigenic drift, even within a single infected individual. In this case, the mutations arise due to the highly error-prone reverse transcriptase involved in the replication of the HIV genome.

Almost unique to the influenza virus is its ability to undergo **antigenic shift**. The influenza virus genome exists as eight separate single-stranded RNA segments, each of which encodes a single viral protein. With such a genetic structure, two different influenza strains that simultaneously infect a single host cell can undergo a re-assortment (sometimes inaccurately called "recombination") of their genomic segments (**Fig. 13-4**). Virus particles containing new combinations of parental RNA suddenly arise, dramatically changing the spectrum of protein epitopes presented to the immune system. This antigenically novel flu virus is safe from antibodies and CTLs raised during previous exposure or vaccination, and so rapidly becomes entrenched in vulnerable hosts constituting a pandemic (see **Box 13-2**).

An *epidemic* involves an increased frequency of disease in one location. A *pandemic* is unrestricted geographically and leads to a global epidemic of disease.

NOTE: Clinical immunologists define a particular antigenic shift of an influenza virus by the identity of its 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. The new strains that result from antigenic shift are often referred to as "influenza virus subtypes."

iii) Interfere with Antigen Presentation

Antigen processing pathways offer many opportunities for a virus to sabotage immune responses, and a given virus can interfere at more than one step.

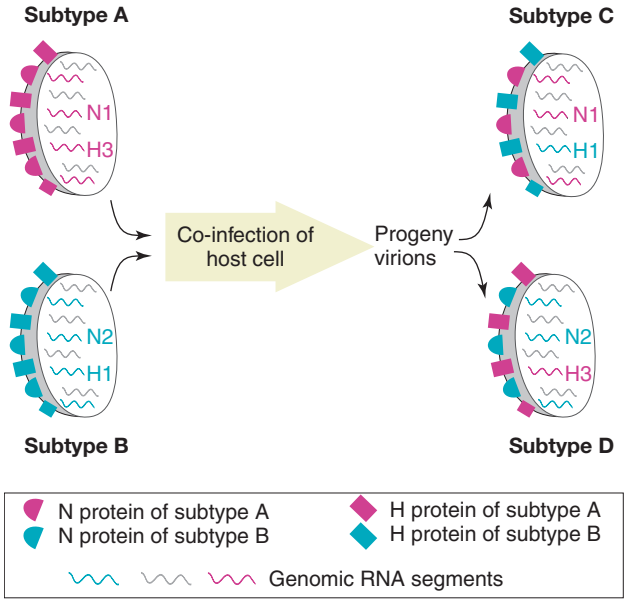

Fig. 13-4
Principle of Antigenic Shift

The eight RNA segments of the influenza virus genome can re-assort if two different viral subtypes infect the same cell. Progeny virions acquiring various combinations of parental segments may express new constellations of proteins. In this *hypothetical* example, influenza virus subtypes A (H3N1) and B (H1N2) have contributed assorted RNA fragments that result in new progeny virus subtypes C (H1N1) and D (H3N2). Gray RNA segments encode internal viral proteins.

Box 13-2 The H1N1 Pandemic of 2009

The antigenic drift that routinely generates a new influenza virus every year is usually responsible for 3–5 million cases of severe illness worldwide and 250,000–500,000 deaths. In contrast, antigenic shifts are responsible for much more serious pandemics, as exemplified by three widespread influenza outbreaks in 1918 (H1N1), 1957 (H2N2), and 1968 (H3N2) that caused tens of millions of deaths around the globe. In this century, a global pandemic of H1N1 influenza began in April 2009. Although the exact series of genetic re-assortment events that led to the emergence of the 2009 H1N1 subtype remains undefined, it is clear that a progeny virus emerged with a hemagglutinin protein to which much of the human population was immunologically naïve. This antigenic shift combined with modern air travel allowed the novel influenza virus subtype to spread very rapidly and efficiently around the world. The potential severity of the situation caused the World Health Organization to issue a global alert, stating that the event was a "Public Health Emergency of International Concern." Fortunately, the mortality associated with this outbreak was far less than in previous pandemics, with global fatalities estimated in the tens of thousands rather than millions. Significantly, older individuals who had survived one or more of the 20th century pandemics appeared to be immune or at least somewhat resistant to the 2009 H1N1 virus. The mechanism mediating this type of cross-subtype protection after an antigenic shift is not well understood. Some immunologists believe that previous infection with the 1918 virus (for example) may have invoked some degree of long-lived protection based on immune responses to more highly conserved proteins in the virus core, such as the viral nucleoprotein or matrix protein. This hypothesis remains under investigation.

a) MHC Class I-Mediated Antigen Presentation

Different viruses avoid activating $CD8^+$ T cells in different ways. Adenovirus blocks MHC class I synthesis in infected cells. CMV and VSV infect cells that normally have very low MHC class I expression. CMV also expresses a protein that induces deglycosylation and degradation of newly synthesized MHC class I chains. A different CMV protein associates with mature peptide–MHC class I structures that do make it to the cell surface and blocks recognition by $CD8^+$ T cells. EBV produces viral proteins that resist proteolysis, meaning that peptides capable of fitting into the MHC binding groove are not easily generated. EBV also downregulates the expression of the TAP antigen transporter and so reduces peptide loading. 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. HIV produces a multifunctional protein called Nef that is able to bind simultaneously to host clathrin proteins and the cytoplasmic tails of MHC class I molecules on the surface of the host cell. This Nef-mediated physical connection between MHC class I and clathrin forces the internalization and lysosomal degradation of the MHC class I molecule.

b) MHC Class II-Mediated Antigen Presentation

Viruses have also evolved myriad ways to avoid activating $CD4^+$ T cells. Rabies virus preferentially infects neurons but is very slow to lyse these cells, meaning that viral antigens are not easily collected by APCs until well after the virus has entered the body. The adaptive response to rabies is thus delayed. CMV and adenovirus both synthesize proteins that inhibit the intracellular signaling pathways required for MHC class II expression. Other viruses express proteins that bind to MHC class II molecules and target them for proteasomal degradation. Still other viruses interfere with MHC class II presentation after the MHC molecule has entered the endocytic system. For example, a CMV protein competes with invariant chain for binding to MHC class II, and certain HPV proteins and the HIV Nef protein disrupt the acidification of the endosomal compartments necessary for peptide generation. As it can for MHC class I, HIV Nef can induce the internalization and lysosomal degradation of cell surface MHC class II molecules.

iv) Fool NK Cells

A virus that causes its host cell to downregulate MHC class I draws the attention of NK cells. CMV therefore expresses a viral homolog of MHC class I that engages NK inhibitory receptors and fools the NK cell into thinking it has detected normal MHC class I. The NK cell is not activated, and the infected host cell is not lysed. CMV also upregulates expression of the non-classical MHC class I molecule HLA-E, which can bind to NK inhibitory receptors. In contrast, the fast-replicating West Nile virus (WNV) upregulates the expression of classical host MHC class I molecules, striving to neutralize NK cells and complete its reproduction before CTLs are generated.

v) Interfere with DCs

Several viruses interfere with DC functions and thus derail T cell responses. Human T cell leukemia virus-1 (HTLV-1) infects DC precursors and prevents their differentiation into immature DCs, blocking the initiation of T cell activation. T cells that interact with the infected DCs are then infected themselves. HSV-1 and vaccinia virus infect immature DCs and block DC maturation, whereas other poxviruses induce the apoptotic death of DCs. Measles virus upregulates the expression of FasL on an infected DC, forcing it to kill any Fas-bearing T cells it encounters. Measles virus can also cause DCs to form large aggregates called *syncytia* in which the virus replicates freely and DC maturation is stymied. Ebola virus infects DCs by binding to the DC lectin DC-SIGN, and this association sequesters the virus within these cells. When CMV infects a DC, the DC becomes tolerogenic so that it anergizes, rather than activates, any naïve T cell it encounters. CMV and herpesviruses also block expression of the chemokine receptor CCR7 by DCs, preventing them from following chemokine gradients into the secondary lymphoid tissues.

vi) Interfere with Antibody Functions

Some viruses are able to interfere directly with production or effector functions of antiviral antibodies. Measles virus expresses a protein that has a negative regulatory effect on B cell activation. An attack by HSV-1 causes the infected host cell to express a viral version of FcγR that binds to IgG molecules complexed to viral antigen. The Fc portion of the antibody is rendered inaccessible by this binding so that neither ADCC nor classical complement activation can be triggered.

vii) Avoid Complement

Viruses use many of the same mechanisms as other pathogens to avoid complement-mediated destruction. Some poxviruses and herpesviruses secrete proteins that block formation of the alternative C3 convertase. Many viruses increase a host cell's expression of the RCA proteins that regulate complement activation, preventing the infected cell from undergoing MAC-mediated lysis. Other viruses express viral homologs of RCA proteins that block MAC-mediated destruction of the virion itself. Still other viruses bud through the host cell membrane and acquire its RCA proteins. The RCA proteins DAF and MIRL are acquired by HIV and vaccinia virus in this way.

viii) Interfere with Host PRRs

Like bacteria, some viruses can thwart innate leukocyte activation by interfering with PRR functions. Vaccinia virus produces proteins that either antagonize kinases or bind to adaptor proteins in TLR signaling pathways, suppressing the activation of host cell transcription needed for antiviral responses. Hepatitis C virus (HCV) synthesizes a protease that cleaves the TLR signaling mediator TRIF, thereby blocking production of IFNs. Paramyxoviruses (like measles virus) produce a protein that associates with the RLR RIG-1, inhibiting the induction of IFNβ production by viral dsRNA. Adenovirus avoids activating TLR9 by having a genome low in CpG motifs. KSHV produces an E3 ubiquitin ligase that promotes the proteasomal degradation of a factor that is needed for TLR-triggered production of IFNs.

ix) Counteract the Antiviral State

Several viruses have developed intricate mechanisms that disrupt the antiviral state. EBV expresses a soluble receptor for a growth factor essential for macrophage secretion of IFNs. In the absence of this growth factor, insufficient IFNs are produced to trigger and maintain the antiviral state. When HSV infects a cell that has already established the antiviral state, the virus expresses proteins that reverse the associated translational block, allowing viral protein synthesis to resume. Vaccinia virus and HCV also synthesize proteins that disrupt the metabolic and enzymatic events needed to maintain the antiviral state. Adenovirus expresses proteins that interfere with the activity of the host's transcription factors. KSHV produces proteins that are homologous to host transcription factors but do not permit transcription of the genes required to establish the antiviral state.

x) Manipulate Host Cell Apoptosis

Host cell apoptosis prior to completion of replication spells viral doom. Host cell apoptosis is most commonly induced by CTL degranulation, Fas/FasL interaction, or the binding of TNF to TNFR. In addition, an infected cell will sometimes be triggered to undergo "altruistic" apoptosis (death for the good of the host) by a mechanism such as **ER stress**. ER stress results when the ER machinery of a host cell is overheated by having to pump out large quantities of viral proteins. Complex viruses with large genomes have developed ways of blocking various steps of these death-inducing pathways. Adenovirus synthesizes a multiprotein complex that induces the internalization of Fas and TNFR, removing these death receptors from the cell surface and forestalling apoptosis induced by an encounter with FasL or TNF. Several poxviruses express homologs of TNFR that act as decoy receptors for TNF and related cytokines. Adenoviruses, herpesviruses and poxviruses express multiple proteins that inhibit the enzymatic cascade necessary for apoptosis. Many viruses can increase intracellular levels of host cell survival proteins that normally prevent premature apoptosis. Alternatively, a virus may express a homolog of these survival proteins that counters apoptosis.

xi) Interfere with Host Cytokines

Early in viral infections, host cells are induced to produce copious quantities of cytokines and chemokines that support antiviral responses. Viruses therefore seek to inhibit the production or action of these molecules, or their receptors. Some poxviruses alter the local cytokine milieu and make it less favorable to the cellular cooperation that underpins an immune response. Both KSHV and adenovirus express proteins that inhibit IFN-inducible gene transcription, whereas certain poxviruses express a protein that blocks IL-1 production. Herpesviruses downregulate cytokine receptor expression, and CMV disrupts the transcription of chemokine genes. Vaccinia virus secretes IFN receptor homologs that intercept IFNα and IFNγ molecules. Poxviruses synthesize a chemokine homolog that binds to chemokine receptors on host cells but blocks the chemotaxis of lymphocytes, macrophages and neutrophils.

Inhibition of IL-12 production is a major goal of many viruses since this cytokine is crucial for Th1 differentiation and thus the antiviral cell-mediated immune response.

EBV synthesizes a homolog of IL-12 that may competitively inhibit the activity of host IL-12. EBV also produces a homolog of IL-10 that suppresses IL-12 production by macrophages and IFNγ production by lymphocytes. The binding of measles virus to certain host cell receptors can also block IL-12 synthesis.

xii) Express Inhibitory miRNAs

Recent studies have revealed that many herpesviruses and some polyoma viruses express microRNA (miRNA) molecules which are not immunogenic themselves but have profound effects on antiviral immunity. Some miRNAs inhibit the expression by infected host cells of viral proteins that furnish immunodominant epitopes. Without presentation of these viral epitopes, CTLs cannot recognize infected host cells and do not kill them, allowing the virus to persist. Similarly, other miRNAs block the expression of the NK activatory ligand MICB by an infected host cell. In the absence of the MICB molecules needed to bind to the NK activatory receptor NKG2D, the NK cell may not receive sufficient activatory signaling to overcome NK inhibitory signaling; the infected host cell is spared. Still other viral miRNAs regulate the activities of innate and adaptive leukocytes by inducing or suppressing host cell expression of various cytokines and chemokines. For example, viral miRNAs may block host cell production of the chemokines IL-8, MCP-1 and/or CXCL11, preventing the recruitment of neutrophils, macrophages and activated T cells that could eliminate the threat. Other viral miRNAs block the expression of molecules that repress IL-10 synthesis, promoting the production of this immunosuppressive cytokine. Similarly, viral miRNAs that inhibit the expression of molecules blocking IL-6 expression have the effect of promoting regulatory T cell differentiation and thus the downregulation of antiviral T cell responses. Over 200 immunomodulatory viral miRNAs have been identified to date and more are emerging daily.

miRNAs are endogenous non-coding RNA molecules (~22 nucleotides in length) that are complementary to sequences in mRNAs and inhibit their translation by binding to them.

E. Immunity to Parasites

I. Disease Mechanisms

Parasites are among the biggest killers in the pathogen pantheon. Parasites include unicellular protozoans and multicellular helminth worms, both of which live within a host (*endoparasites*), as well as arthropods like ticks, fleas, lice and mites, which attach themselves to the skin or hair follicles on the exterior of a host (*ectoparasites*). These scourges claim millions of lives every year, particularly in developing countries. Some protozoans replicate extracellularly, whereas others replicate intracellularly. Helminth worms reproduce inside a host's body but outside its cells, or outside the host entirely in a location (like a water source) where access to a host is easy. Growth and maturation of the worm then occur within the host, often causing severe and long-term damage to tissues and organs. Some ectoparasites complete their entire life cycle on the host surface, whereas others attach only to feed and then detach. Other ectoparasites initially deposit their eggs on the host's skin, but the eggs later detach and mature in soil or water.

Many parasites have multistage life cycles, and each stage of a parasite may be able to infect a different host species. Parasites also frequently use vectors to infect their ultimate hosts, or serve as vectors for other types of pathogens. For example, humans contract malaria through the bite of an *Anopheles* mosquito infected with the protozoan parasite *P. falciparum*. Fleas are a vector for the Gram-negative bacterium *Yersinia pestis*, which causes bubonic plague. Parasites that do succeed in establishing an infection inside an individual may go through various life cycle stages, some of which may be intracellular and others extracellular. All these factors can create a considerable problem from a public health point of view, because a parasite that continually changes form and/or makes use of an invertebrate or animal vector is much harder to control than a pathogen that infects humans only. Both cell-mediated and humoral immunity must often be mobilized to conquer parasites. Examples of diseases caused by protozoans, helminth worms and ectoparasites are given in **Tables 13-7, 13-8** and **13-9**, respectively.

TABLE 13-7 Examples of Parasitic Protozoans and the Diseases They Cause

Pathogen	Disease
Entamoeba histolytica	Enteric disease
Leishmania donovanii	Leishmaniasis in viscera
Leishmania major	Leishmaniasis in face, ears, skin
Plasmodium falciparum	Malaria
Toxoplasma gondii	Toxoplasmosis
Trypanosoma brucei	African sleeping sickness
Trypanosoma cruzi	Chagas disease

TABLE 13-8 Examples of Parasitic Helminth Worms and the Diseases They Cause

Pathogen	Disease
Ascaris	Ascariasis
Cestoda	Tapeworms
Echinococcus	Alveolar echinococcosis
Onchocerca	African river blindness
Schistosoma	Schistosomiasis
Trichinella	Trichinosis
Wuchereria	Elephantiasis

TABLE 13-9 Examples of Ectoparasites and the Diseases They Cause/Transmit

Organism	Role in Disease	Disease Caused
Acari (ticks, some mites)	Pathogen Vector	Dermatitis Lyme disease
Cimicidae (bedbugs)	Pathogen	Skin rashes
Demodex (eyelash mites)	Pathogen	Blepharitis, dermatitis
Hippoboscoidea (tsetse fly)	Vector	Elephantiasis and sleeping sickness
Oestridae (bot flies)	Pathogen	Myiasis
Phthiraptera (lice)	Pathogen	Pediculosis
Sarcoptes scabiei (mites)	Pathogen	Scabies
Siphonaptera (fleas)	Pathogen Vector	Itching, rash

II. Immune Effector Mechanisms

Different parasites evoke different types of immune responses, depending on the size and cellularity of the invader and its life cycle. In general, protozoan parasites tend to induce Th1 responses, while helminth worm infections and attacks by ectoparasites are usually handled by Th2 responses.

i) Defense against Protozoans

a) Induced Innate Defense

Many protozoan components act as PAMPs for TLRs. For example, elements of *Trypanosoma*-derived mucins, phospholipids and genomic DNA bind to TLR2, TLR4

or TLR9, respectively. Certain stages of *Plasmodium* species produce PAMPs that can activate pDCs to produce IFNs in a TLR9-dependent fashion. While some human TLR4 and TLR9 SNPs are associated with an increased risk of developing severe malaria following *Plasmodium* infection, individuals expressing particular TLR1 or TLR6 SNPs develop only mild disease.

Complement activation via the MBL-induced lectin pathway is also important for fighting *P. falciparum* and other *Plasmodium* species that cause malaria. Recent work has identified various MBL SNPs associated with different malarial states in infected individuals, including asymptomatic infection or resistance to infection entirely. Similarly, different SNPs of the complement receptor CR1 or the acute phase protein CRP affect both the frequency of malarial episodes in an infected individual as well as total parasite counts.

NOTE: Although mice genetically deficient for a single TLR do not show increased susceptibility to protozoan parasite infections, animals that lack the TLR signaling adaptor MyD88, which transduces signaling downstream of all TLRs except TLR3, are highly susceptible to infection by *Toxoplasma gondii*, *Trypanosoma cruzi* and *Leishmania major*. In contrast, humans expressing a certain polymorphism of the MyD88-like adaptor protein MAL, which transduces TLR2/TLR4 signaling, are protected against both malaria and Chagas disease (caused by *T. cruzi* infection). This observation suggests a correlation between this polymorphism and increased adaptor function.

b) Humoral Defense

All the effector mechanisms ascribed to antibodies for defense against extracellular bacteria (refer to **Fig. 13-1**) apply to defense against small extracellular protozoans. Antiparasite antibodies mediate neutralization, opsonized phagocytosis, and/or classical complement activation. Larger extracellular protozoans can be dispatched by ADCC mediated by neutrophils and macrophages.

c) Th1 Responses, IFNγ and Macrophage Hyperactivation

The Th1 response is critical for antiprotozoan defense because Th1 effectors are key sources of the IFNγ needed to drive macrophage hyperactivation. Like many intracellular bacteria, many protozoan parasites (e.g., *L. major*) infect or are taken up by macrophages but are not destroyed within ordinary phagosomes. Only in hyperactivated macrophages are sufficient levels of ROIs and RNIs produced to efficiently kill such parasites. In addition, TNF secreted by hyperactivated macrophages plays an important role in the control of protozoans that are still in the extracellular milieu. If all else fails and the hyperactivated macrophages cannot clear the parasite, a granuloma is formed that encompasses the infected host cells and walls off the invader.

IFNγ has several other antiprotozoan effects. This cytokine (1) is directly toxic to various forms of many protozoans; (2) stimulates IL-12 production by DCs and macrophages, which in turn triggers additional IFNγ production by NK and NKT cells; (3) induces iNOS expression in infected macrophages, resulting in the production of intracellular NO that eliminates either the parasite itself or the entire infected cell; (4) upregulates the expression of enzymes important for phagosome maturation; and (5) upregulates Fas expression on the infected macrophage surface, rendering the macrophage susceptible to Fas-mediated apoptosis when it contacts a FasL-expressing T cell. Because Th2 cytokines such as TGFβ, IL-4, IL-10 and IL-13 inhibit IFNγ production and suppress iNOS, individuals that preferentially mount Th2 responses instead of Th1 responses are highly susceptible to diseases caused by protozoan parasites.

d) CTLs and γδ T Cells

If a protozoan parasite escapes from a macrophage phagosome into the cytosol of a host cell, parasite antigens may enter the endogenous antigen processing system such that antigenic peptides are presented on MHC class I. The infected host cells then become targets for CTLs. However, perforin/granzyme-mediated cytolysis is not very effective against acute protozoan infections, and it is CTL secretion of IFNγ that is this

cell type's greatest contribution to the antiprotozoan response. Similarly, IFNγ secretion by activated γδ T cells can bolster the body's defenses during the early stages of protozoan infections. Perforin/granzyme-mediated cytotoxicity becomes important for controlling chronic stages of protozoan infections.

ii) Defense against Helminth Worms

a) Induced Innate Defense

The investigation of mechanisms of innate defense against large, multicellular helminth worms is in its early stages. At least in mice, TLR4 is important for fighting the blood-dwelling trematode *S. mansonii.* Wild type mouse macrophages incubated with preparations of *Schistosoma* larvae are stimulated to produce IL-6, IL-12 and IL-10, but the production of the latter two cytokines is lost in macrophages from TLR-4-deficient mice. Studies are under way to determine the importance of TLR signaling for antihelminth worm responses in humans.

b) Th2 Responses and Humoral Defense

While Th1 responses are needed to combat protozoan parasites, Th2 responses are vital for eliminating helminth worms. For example, humans naturally resistant to *S. mansonii* express high levels of Th2 cytokines, whereas individuals susceptible to this worm exhibit increased concentrations of Th1 cytokines. The antihelminth Th2 response involves IgE, mast cells, basophils and eosinophils, a combination that does not contribute significantly to defense against any other type of pathogen. Activated $CD4^+$ T cells are also critical for antihelminth defense because these cells differentiate into effectors supplying the Th2 cytokines and CD40L contacts required for isotype switching to IgE by B cells (**Fig. 13-5, #1**). The antiparasite IgE antibodies synthesized by the B cells enter the circulation and "arm" mast cells and basophils by binding to cell surface FcεRI. When a worm antigen engages the cell-bound IgE, the degranulation of the mast cells and basophils is triggered in close proximity to the parasite (**#2**). Histamine released by the mast cells and basophils causes the contraction of host intestinal and bronchial smooth muscles such that the parasite is shaken loose from its grip on the mucosal surface and expelled from the body. Histamine and other proteins synthesized by mast cells and basophils are also directly toxic to some helminth parasites. In addition, the vasodilation and increased vascular permeability induced by histamine allow an influx of leukocytes and circulating antibodies into the area. Circulating IgE directed against worm surface molecules may bind directly to the pathogen, attracting the attention of eosinophils expressing FcεRI molecules. The binding of the worm-bound IgE to eosinophil FcεRs triggers eosinophil degranulation and the release of substances that work directly and indirectly to kill the worm (**#3**). Some molecules degrade the skin of the worm, allowing neutrophils and other leukocytes to penetrate into its underlying tissues. These cells may also degranulate and release additional toxic proteins and peptides that kill the worm. Other molecules contained in eosinophil granules stimulate mast cells to degranulate.

Th2 cytokines are critical for eliminating helminth worms. IL-4 produced by basophils and Th2 effectors is the main cytokine driving isotype switching in B cells to IgE. IL-5 produced by Th2 cells strongly promotes the proliferation, differentiation and activation of eosinophils, and supports the differentiation of plasma cells that have undergone isotype switching to IgA production (**Fig. 13-5, #4**). Secretory IgA coats the mucosae and fends off further parasite attachment. IL-4 and IL-13 produced by basophils and Th2 cells suppress macrophage production of IL-12, inhibiting IFNγ production and hence the development of a Th1 response (which would be largely ineffective). IL-13 also stimulates bronchial and gastrointestinal expulsion responses.

iii) Defense against Ectoparasites

Ectoparasites are often arthropods that attack the exterior surface of a host. For example, the common tick is the carrier of the extracellular bacterium *Borrelia burgdorferi* responsible for Lyme disease. The bacteria are introduced into the host when the tick bites him/her to obtain a blood meal. Large numbers of basophils, eosinophils and mast cells

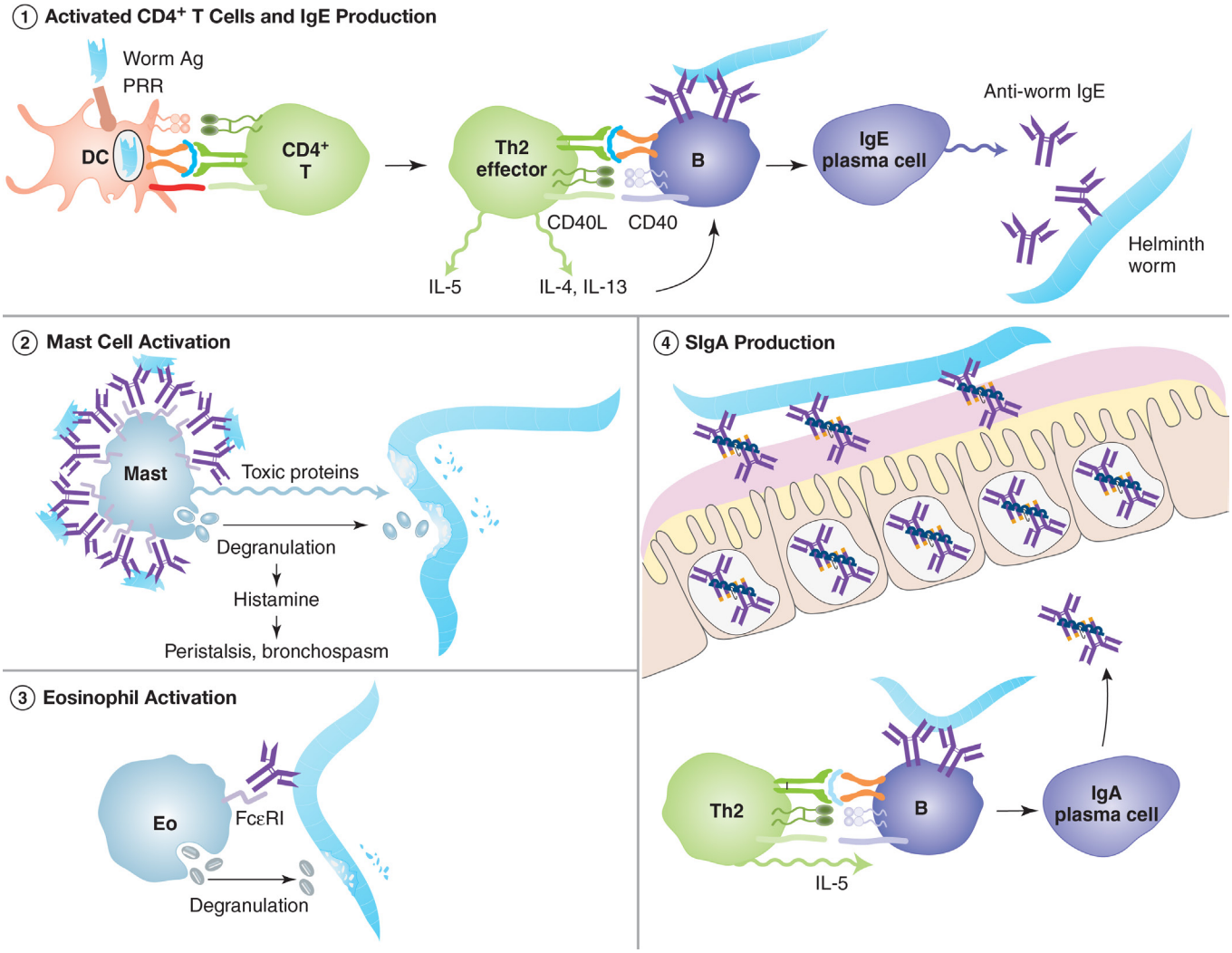

Fig. 13-5
Major Mechanisms of Immune Defense against Helminth Worm Parasites

(**1**) DCs presenting worm peptides induce CD4+ Th2 cell differentiation. Th2 effectors produce cytokines that induce activated B cells to undergo isotype switching to IgE. (**2**) Mast cells (and basophils; not shown) pre-armed with antiparasite IgE are activated by worm antigens and release histamine and toxic proteins. (**3**) Activated eosinophils bind to worm-bound antibodies via their FcεRIs and degranulate, releasing molecules that directly damage the worm surface. (**4**) IL-5 produced by Th2 cells induces isotype switching to IgA in mucosal B cells specific for worm antigens. Secretory IgA (SIgA) blocks the worm from gaining a foothold on the mucosal surface.

accumulate at the bite site to repel both the attacking bacteria and the tick. It is thought that when mast cell degranulation releases substances that increase vascular permeability, ticks have greater difficulty in locating host blood vessels. Some ectoparasites are countered by the same strategies effective against helminth worms. Antipathogen IgE bound to the surface of basophils and mast cells is critical for host defense against such invaders. For example, humans who lack adequate numbers of basophils and eosinophils develop scabies, a severe, itchy rash caused by the mite *Sarcoptes scabiei*. Much remains to be determined about the molecular details of immune responses to ectoparasites.

NOTE: The involvement of Th2 responses in defense against ectoparasites came from the unexpected finding of increased *Demodex* skin infections in mice lacking both CD28 and STAT6. CD28 is a key costimulator of Th cell activation, and STAT6 is the transcription factor required for IL-4 production by these cells.

III. Evasion Strategies

A parasite that has a multistage life cycle enjoys a wealth of opportunities to thwart the immune response. Several evasion strategies used by protozoa and/or helminth worms are described in the following sections and summarized in **Table 13-10**.

i) Avoid Antibodies

Different parasites employ different strategies to avoid antibodies. Protozoans with multiple life cycle stages can take advantage of the escape offered by antigenic variation. Just as the host mounts a humoral response to epitopes associated with one stage of the parasite, the organism may take on a totally different form and present a whole new panel of epitopes to the host's immune system. A lag in defense ensues while antibodies are produced to counter the new set of antigens. Other protozoans take a more direct approach. *L. major* hides from antibodies by sequestering itself within host macrophages. Some *Schistosome* helminths disguise themselves by acquiring a coating of host glycolipids and glycoproteins. The dense "forest" created by these host molecules blocks antibodies from binding to parasite surface antigens. Other helminths repel antibody attack by shedding parts of their external membranes, ejecting the immune complex of the parasite antigen and host antibody. Still other helminths produce substances that digest antibodies.

Trypanosoma brucei, the causative agent of African sleeping sickness, confounds antibodies by rapid antigenic variation. This pathogen can spontaneously modify its expression of its *variable surface glycoprotein (VSG),* the molecule that is normally the main target of humoral responses to this parasite. There are hundreds of VSG genes but each trypanosome expresses only one VSG gene at a time. However, the trypanosome regularly shuts down expression of the first VSG gene and activates another, resulting in an altered glycoprotein coat that may not be recognized by antibodies raised against the first VSG protein. The trypanosomes are therefore able to outpace the immune system's ability to adapt to the change in VSG antigens, buying the time the organisms need to penetrate the blood–brain barrier and enter the central nervous system (CNS). This ability of *T. brucei* to artfully evade the immune system accounts for the near 100% mortality of untreated African sleeping sickness.

TABLE 13-10 Evasion of the Immune System by Parasites

Immune System Element Thwarted	Parasite Mechanism
Antibodies	Have a multistage life cycle that furnishes antigenic variation Hide in macrophages Modify parasite surface proteins to cause antigenic variation Acquire host surface proteins that block antibody binding Shed parasite membranes bearing immune complexes Secrete substances that digest antibodies
Phagocytosis	Block fusion of phagosome to lysosome Escape from phagosome into cytoplasm Block respiratory burst Lyse resting phagocytes
Complement	Degrade attached complement components or cleave Fc portions of membrane-bound antibodies Force complement component exhaustion Express homologs of RCA proteins
T cells	Inhibit Th1 response by promoting IL-10 production and decreasing IL-12 and IFNγ production Secrete proteins inducing hyporesponsiveness or tolerance of T cells Interfere with DC maturation and macrophage activation Induce downregulation of surface MHC class I and II

ii) Avoid Phagolysosomal Destruction

Helminth worms are in no danger of being captured by phagocytosis, but many protozoans have developed means of avoiding such destruction. For example, some intestinal protozoans lyse resting granulocytes and macrophages and thus minimize their chances of being engulfed in the first place. *T. gondii* blocks the fusion of macrophage phagosomes to lysosomes. *T. cruzi* enzymatically lyses the phagosomal membrane prior to lysosomal fusion and escapes to the cytoplasm of the host cell. *L. major* often remains in the phagosome but interferes with the respiratory burst.

iii) Avoid Complement

Both protozoans and helminths can take steps to avoid complement. Certain members of both groups can proteolytically remove complement-activating molecules that have attached to their surfaces, or cleave the Fc portions of parasite-bound antibodies. For example, *L. major* can induce the release of the entire complement terminal complex from its surface. Other parasites secrete molecules that force continuous fluid phase complement activation, thereby exhausting complement components. Still other parasites express a molecule that functionally mimics the mammalian RCA protein DAF.

iv) Interfere with T Cells

Members of both the protozoan and helminth groups have evolved ways of manipulating the host T cell response to favor parasite survival. For example, *P. falciparum* can promote Th cell secretion of IL-10 rather than IFNγ, resulting in downregulation of MHC class II expression and inhibition of NO production. This pathogen also expresses molecules that cause the red blood cells it infects to indirectly interfere with macrophage activation and DC maturation. *L. major* expresses molecules that can bind to CR3 and FcγR molecules on macrophages and reduce IL-12 production by these cells. The Th1 response that would kill the protozoan is thus inhibited. Nematode hookworms secrete several proteins that induce hyporesponsiveness or even tolerance in host T cells. This state of immunosuppression allows great masses of worms to accumulate in the infected host. Other filarial worms induce the APCs with which they come into contact to decrease their surface expression of MHC class I and II, and to also downregulate other genes involved in antigen presentation. The APCs cannot then participate effectively in T cell activation.

Focus on Relevant Research

"Immunodiagnosis of *Taenia solium* taeniosis/cysticercosis" by Deckers, N. & Dorny, P. (2010) *Trends in Parasitology* 26, 137–144.

Although unpleasant to deal with for both patient and physician alike, infection by the pork cestode intestinal tapeworm *Taenia solium* is relatively easy to diagnose, and adult worms that have evaded immune destruction are usually eliminated with antihelminthic medications. A much more serious condition arises when tapeworm cysts lodge in non-intestinal tissues such as the muscle, eye or brain. This condition, known as *cysticercosis*, can be extremely difficult to diagnose and may cause significant tissue damage. Arguably the most severe form of cysticercosis is *neurocysticercosis* (cysts in the brain), which can result in convulsions, permanent brain damage, or death. Neurocysticercosis caused by *T. solium* is the most common parasitic disease of the CNS worldwide, and early and accurate diagnosis of this infection could significantly reduce its global morbidity and mortality. This article by Deckers and Dorny reviews the numerous laboratory tests currently available and under development for the diagnosis of various forms of cysticercosis. The authors also describe several candidate *T. solium* molecules that may prove useful for the development of future diagnostic tools. Most of these tests are immunologically based and include techniques such as radioimmunoassay, hemagglutination, complement fixation, and ELISA. The principles of these serological techniques are illustrated in Appendix F.

F. Immunity to Fungi

I. Disease Mechanisms

Fungi are either unicellular and grow as discrete eukaryotic cells (like yeast), or are multicellular and grow in a mass (*mycelium*) of filamentous processes (*hyphae*). *Dimorphic* fungi adopt a unicellular form at one stage in their life cycle and a multicellular form at another stage. *Conidia* are haploid, non-motile fungal spores that are formed under unfavorable nutrient conditions. All fungal cells have a cell wall like bacteria but also a cell membrane like mammalian cells. Although many fungi live most of their lives in the soil, some live commensally on the topologically external surfaces of the human body. Some fungi are *dermatophytes*, filamentous fungi that infect only the skin, hair and nails. Most fungal species are not harmful to healthy humans, but when a fungus succeeds in invading the body, it usually heads for the vascular system of the target tissue. Invasion of blood vessels by a growing fungus can choke off the blood supply to the host's organs.

Fungi have recently become a more significant clinical threat due to the advent of modern protocols for organ transplantation, treatment of autoimmunity, implantation of medical devices, and chemotherapy. All these procedures call for or result in suppression of the patient's immune system, so that fungi which would normally not succeed in establishing an infection are able to do so; that is, the patients contract opportunistic infections. In particular, species of *Aspergillus*, *Candida* and *Cryptococcus* fungi have become prominent threats to immunocompromised individuals. Patients may also present with infections by one of the *Mucormycotina* group of filamentous fungi, which launch life-threatening attacks on the brain and sinuses. Diseases caused by fungal infections are generally called *mycoses*, and examples of several are given in **Table 13-11**.

Cancer chemotherapy and organ transplantation are discussed in Chapters 16 and 17, respectively.

NOTE: A rising concern among clinical immunologists studying fungal infections is the projected impact of global warming. With climate change will come two potential threats to the currently balanced relationship between most fungi and their mammalian hosts. First, a warmer environment will allow new fungal species to survive in previously hostile geographic areas, increasing the number of threats faced by vulnerable individuals. Second, some researchers believe that the planet is warming faster than mammals can evolve to maintain the usual difference between their own body temperature and the generally lower temperature of their surroundings. Normally, this temperature differential contributes to the resistance of healthy mammals to most fungi. As a result, global warming may open up new colonization opportunities for fungal pathogens.

II. Immune Effector Mechanisms

i) Induced Innate Immunity

Mechanisms of induced innate immunity are very important for controlling fungal infections. TLR2, TLR4, DC-SIGN, Dectin-1, Dectin-2, CR3, MBL and MR all recognize PAMPs supplied by molecules in fungal cell walls or on the fungal cell surface. These

TABLE 13-11 Examples of Fungi and the Diseases They Cause

Pathogen	Disease
Aspergillus species	Respiratory infections, acute and chronic pneumonias
Blastomyces dermatitidis	Blastomycosis; skin lesions, acute and chronic pneumonias
Candida species	Yeast infections, vaginitis, cystitis
Cryptococcus neoformans	Meningitis, pneumonia
Histoplasma capsulatum	Histoplasmosis; lesions in the lung
Mucormycotina	Mucormycosis; lesions in the brain, sinuses and lung; eye swelling
Paracoccidioides brasiliensis	Ulcerations of mucosae of nose and mouth
Pneumocystis (carinii) jiroveci	PCP pneumonia and lung damage
Dermatophytes	Skin, nail and hair infections

PAMPs include fungal β-glucans, chitins, mannans and oligomannosides. In particular, Dectin-1 and Dectin-2 are CLRs specialized for the recognition of fungal PAMPs. Dectin-1 binds to cell wall β-glucans, whereas Dectin-2 recognizes mannose structures common to the hyphal forms (only) of many fungi. Dectin-1 contains its own ITAM in its cytoplasmic tail, whereas Dectin-2 pairs with the FcRγ signaling chain to transduce intracellular signaling. This signaling results in host cell production of pro-inflammatory cytokines and leukotrienes instrumental in removing the invader. Dectin-1 is widely expressed on DCs, monocytes, macrophages, neutrophils and some T cells. Dectin-2 expression is more restricted, being limited to monocytes and macrophages. The importance of Dectin-1 has been highlighted by recent studies of a family in Holland, some members of which lack Dectin-1 expression and suffer from recurrent fungal infections. Individuals with deficits in the expression of other C-lectin receptors are also unusually susceptible.

A glucan is a polymer of glucose molecules, a chitin is a polymer of N-acetylglucosamine molecules, and a mannan is a chain of mannose molecules added to a protein.

Attack by a fungus often induces a host to assemble the NLRP3 inflammasome that drives IL-1 and IL-18 production. Host cell-derived DAMPs associated with fungal infections include the S100 proteins that bind to RAGE, a PRR introduced in Chapter 3. As was true for protozoan parasites, animals lacking expression of the TLR signaling adaptor protein MyD88 are very vulnerable to fungal infections. Neutrophils and macrophages activated by PRR engagement carry out vigorous phagocytosis and produce powerful antifungal defensins that induce osmotic imbalance in the fungal cells (**Fig. 13-6, #1**). Fungal cells may also trigger their own engulfment by some cell types that are not normally phagocytic, including epithelial and endothelial cells. As well as defensins, activated neutrophils and macrophages secrete copious quantities of chemokines along with IL-1, IL-12 and TNF, which are directly toxic to fungal cells. γδ T cells appear to play a significant role in antifungal defense at the mucosae (**#2**), a hypothesis based on the fact that mice engineered to lack γδ T cells show increased susceptibility to yeast infections. Activated NK cells stimulated by the presence of IL-12 contribute to fungal cell killing via TNF secretion (rather than by natural cytotoxicity) (**#3**). IFNγ produced by NK cells contributes to macrophage hyperactivation that can eventually lead to granuloma formation.

NOTE: Expression of SNPs of many of the molecules associated with antifungal responses, including TLRs, CLRs, cytokines and chemokines, leads to increased susceptibility to fungal infections. For example, a TLR2 SNP that results in increased TNF production but decreased IL-18 and IFNγ secretion by leukocytes is associated with more severe *C. albicans* infections. Similarly, TLR4 and TLR9 SNPs have been linked to *Aspergillus* infections of the lungs. SNPs in Dectin-1 and NLRP3 that impair cytokine production leave the host vulnerable to fungal infections of the mucosae and/or skin. Likewise, SNPs in MBL or MASP that alter the lectin-mediated complement activation pathway allow *Aspergillus* to set up shop, as do SNPs in TNF or TNFR1/2 that decrease their expression. Interestingly, SNPs in IFNγ and IL-4 that *increase* production of these cytokines can dysregulate the adaptive response so much that fungal invasion succeeds. Clinicians may soon be able to use all these SNPs as genetic markers to identify patients who are at high risk for developing fungal diseases if their immune systems are deliberately suppressed. Preventive treatment can then be offered to these individuals.

ii) CD4+ T Cells

T cell responses against fungi are shaped by the DCs activating them, and DCs have demonstrated a remarkable ability to distinguish among various types of fungi based on patterns of PRR engagement. Most DCs that acquire fungal antigens and experience TLR signaling undergo maturation and activate naïve T cells to generate Th1 effectors. These T cells secrete the copious quantities of IFNγ needed to complete macrophage hyperactivation (**Fig. 13-6, #4**). DCs whose MRs and Dectin-1 molecules are engaged by fungal PAMPs are stimulated to produce IL-23 and IL-10, which initiate the generation of Th17 effectors. These cells contribute mainly to protection against fungi attempting to colonize the mucosae, including *C. albicans* and some *Aspergillus* species. Antifungal Th17 cells secrete IL-17 and IL-22 that contribute to the neutrophil recruitment and proliferation crucial for fungal clearance, and support Th1 responses. Both αβ Th17 and γδ Th17 cells have been found in mice experimentally infected with *C. albicans.*

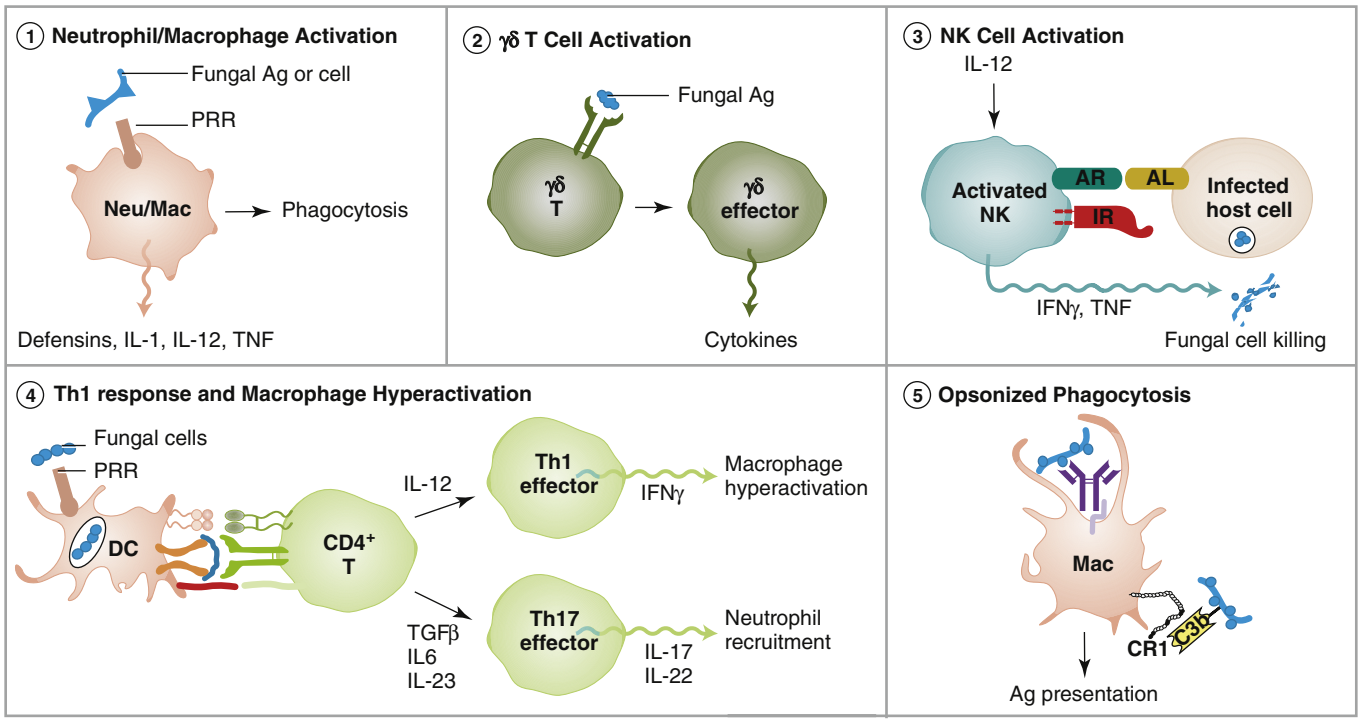

Fig. 13-6
Major Mechanisms of Immune Defense against Fungi

(1) Activated neutrophils and macrophages carry out phagocytosis of fungal cells and secrete antifungal peptides and cytokines. **(2)** Activated mucosal γδ T cells generate effectors that secrete cytokines. **(3)** Activated NK cells kill fungi by secreting cytotoxic cytokines rather than by natural cytotoxicity. **(4)** Fungal PRR ligands activate DCs that drive Th1 or Th17 effector differentiation, leading to either macrophage hyperactivation and granuloma formation or neutrophil recruitment. **(5)** Fungi coated in either antifungal antibody or C3b undergo opsonized phagocytosis by macrophages (and neutrophils; not shown). Note that the structure of the fungal cell wall allows fungal cells to resist MAC-mediated lysis.

Th2 responses are comparatively rare during fungal infections and not very effective. Those patients who respond to fungi with Th2 responses instead of Th1 responses show poor resistance to these pathogens. For example, patients who mount Th1 responses against *Paracoccidioides brasiliensis* experience only mild and transient paracoccidioidomycosis (ulceration of the mucosae in the mouth and nose), whereas those who mount Th2 responses have severe disease that relapses frequently. Interestingly, the female hormone estradiol promotes Th1 responses in this context, likely accounting for the fact that paracoccidioidomycosis occurs much less often in women than in men.

iii) Humoral Defense

Conventional antibodies are thought to contribute in only a limited way to defense against fungi that manage to invade the body. Antibody-mediated opsonization may promote phagocytosis (Fig. 13-6, #5) and thus contribute to the clearance and presentation of fungal antigens. However, antibodies produced by a unique B cell subset called *B-1 cells* are critical for antifungal defense. In Chapters 4 and 5, we described conventional B cell production of antibodies of the IgM, IgG, IgA and IgE isotypes in response to antigen. However, the serum of normal healthy individuals contains IgM antibodies that are pre-existing and generated without the apparent need for exogenous antigen. These proteins, which are called **natural antibodies**, are produced by B-1 cells scattered in the body's periphery rather than concentrated in the bone marrow or secondary lymphoid tissues. Many natural antibodies are specific for the β-glucans and chitins in fungal cell walls.

NOTE: There is a fine balance that must be maintained during inflammatory responses to fungi to ensure host health. Acute inflammation is helpful in getting rid of the invader, but chronic inflammation that fails to clear the pathogen appears to encourage fungal persistence. Severe fungal infections can occur in patients who have started to recover function of their immune systems after immunosuppression has been used to allow transplantation, or after AIDS has been brought under control by anti-HIV drug therapy. These individuals sometimes show clinical signs of a disease labeled *immune reconstitution inflammatory syndrome (IRIS)*. As the immune system starts to reconstitute itself, it mounts an excessive inflammatory response to an opportunistic pathogen such as a fungus that has taken hold during the immunosuppression. Rather than resolving the infection, this inflammation disrupts immune system regulation and compromises fungal clearance, paradoxically making the condition worse.

III. Evasion Strategies

Many fungi adopt different morphological forms at different stages in their life cycle, affording them multiple opportunities to evade immune defense as described in the following sections and summarized in **Table 13-12**.

i) Avoid PRRs

To avoid detection by PRRs, some fungi shift their morphological form from yeast-like to hyphae to reduce the presence of detectable PAMPs. Other fungi take alternative steps to obscure their PAMPs. For example, *C. albicans* expresses a protein that covers the β-glucan molecules in its cell wall, preventing recognition by phagocytes bearing Dectin-1. Similarly, *Histoplasma capsulatum* hides its β-glucan under a layer of α-glucan, which does not bind to Dectin-1. *Aspergillus fumigatus* covers its spores in proteins such as melanin that block recognition of the conidial surface by innate leukocytes. In contrast, *Pneumocystis jiroveci* simply changes the expression of its major surface glycoproteins, confounding both PRR and antibody recognition. Lastly, the cell walls and membranes of some fungi simply lack PAMPs and other structures that might trigger PRR-mediated recognition, forestalling both phagocytosis and the binding of complement components.

Pneumocystis jiroveci is the new name of *Pneumocystis carinii*, the fungus that causes pneumonia in AIDS patients (see Ch. 15).

TABLE 13-12 Evasion of the Immune System by Fungi

Immune System Element Thwarted	Fungal Mechanism
Host PRRs	Shift between different morphological forms Hide lectin-binding cell wall components under another molecular layer Change expression of major surface molecules Have no LPS or peptidoglycan in cell wall
Phagocytosis	Form a very large mass Have a capsule or produce a protein that blocks phagocytosis Detoxify NO or inhibit its production Secrete factors that neutralize the phagosomal environment Alter phagosome maturation Escape a phagocyte by vomocytosis
Complement	Have a cell wall that blocks access to the cell membrane Recruit host RCA proteins to the fungal surface Produce proteases that digest complement components
Th response	Secrete molecules suppressing macrophage cytokine production and B7 expression Secrete molecules inducing ineffective Th2 response rather than Th1 response Secrete molecules blocking APC and/or T cell differentiation or proliferation Activate regulatory T cells
Antibodies	Have a multistage life cycle Have a capsule not easily recognized by antibodies Secrete molecules blocking B cell differentiation, proliferation

ii) Avoid Phagocytes

When species such as *C. albicans* and *A. fumigatus* are present in their multi-nucleate hyphal morphology, they form a mass too large to be captured by phagocytosis. In a similar vein, *Cryptococcus neoformans* forms very large polyploid "titan cells" that are encased in a thick polysaccharide capsule. The sheer size of these cells and the composition of their capsule provide a formidable barrier to phagocytosis. *C. neoformans* also secretes a small protein called App1 that is induced under conditions of low glucose, such as are routinely found in the lung (the primary site of attack of this pathogen). App1 interacts with CR2 and CR3 in such a way as to block macrophage phagocytosis of any fungal cells opsonized by C3d or iC3b, respectively.

"Polyploid" means that more than one set of chromosomes is contained within the nucleus of a single cell.

Even when fungal cells are engulfed by phagocytes, many of these pathogens have ways of enzymatically detoxifying the NO produced by phagocyte iNOS activity. *C. albicans* secretes an unknown inhibitory factor to accomplish this task, whereas *C. neoformans* and *Blastomyces dermatitidis* establish inhibitory intercellular contacts with macrophages. *H. capsulatum* neutralizes phagosomal killing by secreting factors that alter the phagolysosomal environment and render it non-acidic. Other fungi appear to alter intracellular endosomal trafficking such that phagosome maturation is impaired. *C. neoformans* has perfected a novel route that allows it to escape from macrophage phagosomes, and indeed the whole leukocyte, without damage to either cell. The fungal cell secretes molecules that weaken the membrane of a macrophage phagosome, which then fuses with the macrophage plasma membrane. The contents of the phagosome, including the fungal cell, are then expelled from the macrophage into the extracellular milieu by exocytosis. Some immunologists have given this process the enchanting name "vomocytosis."

iii) Avoid Complement

Fungal cells are frequently opsonized by complement products. However, although fungal cells activate the complement cascade, their cell walls and recruitment of RCA proteins render them generally resistant to complement-mediated lysis. For example, *C. albicans* expresses several proteins that can recruit host RCA proteins, including Factor H and C4bp, to the fungal surface. The spores of *A. fumigatus* also produce a Factor H-binding protein. In addition, both *C. albicans* and *A. fumigatus* secrete proteases that can digest numerous host proteins, including the complement components C3b, C4b and C5.

iv) Promote a Less Effective Th Response

Many fungi produce toxins and other molecules that have immunosuppressive effects and/or promote immune deviation to an ineffective Th2 response at the expense of a Th1 response. Fungal ligands that engage certain PRRs on epithelial cells induce them to produce TSLP and IL-25. These cytokines tend to amplify Th2 responses, which are weak against fungi, and also promote iTreg cell generation. The fungus thus gains ground in winning host tolerance to itself. Infection by *C. albicans* induces host cell production of IL-10, as does a switch in fungal cell morphology by *A. fumigatus*. Other fungal molecules inhibit the transcription of genes needed for the differentiation of activated T cells. Still other fungal mediators suppress lymphocyte proliferation or macrophage cytokine production. A polysaccharide in the capsule of *C. neoformans* blocks IL-12 production by monocytes/macrophages, downregulates macrophage B7 expression, and activates regulatory T cells. Both *C. neoformans* and *H. capsulatum* (among other species) also release membrane-bound *exosomes* that contain numerous virulence factors, such as anti-oxidant proteins and capsule biosynthetic enzymes, that help to sustain the fungal infection and blunt the host's response to it. The contents of these so-called "virulence bags" remain under investigation.

v) Avoid Antibodies

The various morphologies a fungus can adopt during its life cycle may result in a constantly changing array of surface epitopes that can confound antibody recognition. In addition, even if an antibody does bind to the fungal capsule, its thick polysaccharide

structure may inhibit subsequent FcR-mediated phagocytosis by innate leukocytes. Some fungi also secrete molecules that inhibit the transcription of genes needed for B cell differentiation or proliferation, or block these processes directly.

G. Prions

Prions are the pathogens that cause *spongiform encephalopathies* (SEs), which are rare, lethal neurodegenerative diseases characterized by lesions that render the brain "sponge-like." The affected host develops dementia and loses motor function control shortly before death. The major human SE diseases are called *variant Creutzfeldt–Jakob disease* (vCJD) and Kuru (the "shaking disease" of Papua New Guinea). Animal SEs include *scrapie* in sheep and *bovine spongiform encephalopathy* (BSE, or "mad cow disease") in cattle. These disorders are associated with the ingestion of infected tissues from an animal suffering from an SE. For example, a cow that consumes cattle feed made from the remains of a contaminated sheep may contract BSE, whereas a human who enjoys a hamburger made from the meat of the infected cow may eventually succumb to vCJD.

I. Disease Mechanisms

Prions are essentially transmissible proteins devoid of nucleic acid. Structurally, a prion is a conformational isomer of a normal mammalian surface glycoprotein. In the original studies of scrapie in sheep, this normal glycoprotein was denoted PrP^{c} (prion protein, cellular) and the altered protein was denoted PrP^{sc} (prion protein, scrapie). PrP^{res} (prion protein, resistant to proteases) is now used to denote the altered protein in any species. When PrP^{res} is introduced into a healthy animal, it acts as a template for the refolding of existing host PrP^{c} molecules into additional copies of PrP^{res}. The disease-causing prion thus effectively "replicates" itself in a mass conversion of the host's PrP^{c} molecules to the PrP^{res} conformation. The misfolded PrP^{res} protein has profoundly altered properties compared to PrP^{c}. As a result, the PrP^{res} protein can enter neurons in the brain and induce the degeneration of this organ 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^{res}.

The description just given is of the infectious form of prion disease, in which 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. However, rare cases of prion disease do arise spontaneously due to a mutation of an individual's PrP^{c} gene that results in production of a PrP^{res} protein. As long as the tissues bearing the PrP^{res} protein are not later ingested by another animal, there is no transmission of the disease. In rare cases, however, the mutation may occur in a germ cell such that the disease is inherited by the affected animal's offspring.

NOTE: Until quite recently, the function of the normal PrP^{c} protein, other than to serve as a template for production of PrP^{res} proteins, was unknown. Studies in the past few years have revealed that PrP^{c} plays roles in several neuronal processes, including cell adhesion, neurite outgrowth, ion channel activity, and excitability. Intriguingly, the normal PrP^{c} protein also appears to mediate the assembly of the toxic oligomers of amyloid-β peptide that accumulate in the brains of Alzheimer's disease patients. These observations raise the possibility that therapeutic mAbs directed against the PrP^{c} domains that promote oligomer assembly might provide an effective treatment for Alzheimer's disease.

II. Immune Effector Mechanisms

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 infectious PrP^{res}

protein, as it is merely a naturally occurring self protein with a modified secondary structure. 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^{res} might be recognized by the BCR of a B cell, the antigen itself cannot act as a Ti immunogen because it has neither the large size nor multivalency needed to activate B cells. Thus, no adaptive responses are mounted against prions. Fortunately, however, new evidence is coming to light indicating that innate immune defense against prions does exist and may help to at least slow the course of SE disease. Mice engineered to lack the transcription factor IRF3, which is important for some TLR signaling pathways, showed faster onset of prion disease than control animals. In cell cultures, cells that were infected with prions and treated to specifically inactivate IRF3 accumulated higher amounts of PrP^{res} protein, whereas cells that were engineered to express abnormally large amounts of IRF3 sustained lower levels of prion infection. Similar results have been found for mice with an inactivating mutation of TLR4. The role of innate immunity in combatting prions remains under investigation.

This brings us to the end of our description of the mechanisms of natural immune defense against pathogens. In the next chapter of this book, we discuss the "manufactured" immunity to pathogens created by vaccination.

Chapter 13 Take-Home Message

- There are six major types of pathogens: extracellular bacteria, intracellular bacteria, viruses, parasites, fungi and prions.
- Innate immunity mediated by neutrophils, NK cells, NKT cells, γδ T cells, complement and microbicidal molecules either foils the establishment of infection or slows it down until adaptive immune mechanisms can target the pathogen more effectively.
- The adaptive elements that will be most effective depend on the nature of the pathogen: extracellular versus intracellular, small versus large, fast- versus slow-replicating.
- Most extracellular entities can be coated in antibody and cleared by antibody- and complement-mediated mechanisms. Parasitic worms are targeted by IgA and IgE antibodies that prevent the worm from anchoring in the host. IgE can trigger the degranulation of mast cells, basophils and eosinophils and the release of mediators that work to expel the worm and degrade its tissues.
- Intracellular bacteria and parasites and replicating viruses must be eliminated by cell-mediated immunity. CTLs, NK cells, NKT cells and γδ T cells secrete cytotoxic cytokines and/or carry out target cell cytolysis. Macrophage hyperactivation and granuloma formation may be needed to confine persistent invaders.
- In general, Th1 and Th17 responses support cell-mediated immunity against internal threats, whereas Th2 responses are needed for humoral responses against external threats.
- Many pathogens have evolved complex strategies to evade the immune response: avoiding recognition; antigenic variation; avoiding or inactivating phagocytosis; shedding or inactivating complement components; acquiring host RCA proteins; cleaving host FcRs; inducing host cell apoptosis; and manipulating the host's immune response or cell cycle.

Did You Get It? A Self-Test Quiz

Introduction and Section A

1) Characterize the six major classes of pathogens.
2) How is disease distinct from infection?
3) What is immunopathic disease?
4) Distinguish between opportunistic and invasive pathogens.
5) Outline two ways each by which the skin and mucosae defend the body's external surfaces.
6) Name four types of leukocytes that mediate subepithelial innate defense and give examples of their effector mechanisms.
7) What is an SNP, and how can it be useful to clinical immunologists?
8) Name three pathogens that induce NLRP inflammasome assembly.

Section B

1) Distinguish between exotoxins and endotoxins.
2) Name two diseases caused by exotoxins.
3) What is endotoxic shock, and why is it considered immunopathic?
4) Describe three ways in which antibodies help protect the body against extracellular bacteria.
5) What is an antitoxin, and what does it do?
6) Describe an extracellular bacterial infection combatted by Th17 cells.
7) Outline two mechanisms each by which extracellular bacteria can evade host PRRs; antibodies; phagocytosis; complement.

Section C

1) Can you define these terms? vector, granuloma
2) How are neutrophils, NK cells, and γδ T cells helpful in combatting intracellular bacteria?
3) Name three TLRs that contribute to defense against intracellular bacteria.
4) Why is macrophage hyperactivation effective against intracellular bacteria?
5) Briefly outline the sequence of cellular and molecular events that lead from an intracellular bacterium successfully gaining a foothold in the body to CTL-mediated elimination of cells infected with that bacterium.
6) How do various subsets of $CD4^+$ Th effectors contribute to defense against intracellular bacteria?
7) Describe how the different forms of leprosy illustrate the importance of Th responses to intracellular bacteria.
8) How can antibodies be useful for the control of intracellular bacteria?
9) Name three types of leukocytes crucial for granuloma formation and outline the role each plays.
10) Outline five mechanisms by which intracellular bacteria can evade phagosomal death.
11) Outline one mechanism each by which intracellular bacteria can avoid host PRRs; antibodies; T cells.

Section D

1) Can you define these terms? acute disease, chronic disease, persistent infection, latency, oncogenic, iNOS, syncytia
2) What is the antiviral state, and how is it induced?
3) How do pDCs and NK cells combat viruses, and why are these cells crucial for early defense?
4) Name three PRRs important for antiviral defense and the PAMP/DAMP each of these PRRs recognizes.
5) Describe three ways in which $CD4^+$ T cells contribute to immune defense against viruses.
6) Describe three ways in which $CD8^+$ T cells contribute to immune defense against viruses.
7) Describe four ways in which antibodies contribute to immune defense against viruses.
8) Describe two mechanisms of latency and outline how viral latency can be reversed.
9) Distinguish between antigenic drift and antigenic shift.
10) Distinguish between epidemic and pandemic.
11) Describe three ways each by which viruses can resist attack by CTLs, $CD4^+$ T cells, and complement.
12) Outline three ways each in which viruses can counteract the antiviral state; interfere with host PRRs; inhibit the induction of host cell apoptosis; interfere with host cell cytokines.
13) Give two ways each in which viruses compromise NK cell, DC and antibody responses.
14) What is ER stress, and how can it be beneficial to a host?
15) Give three examples of how miRNA production can help a virus to persist.

Section E

1) Distinguish between ectoparasites and endoparasites.
2) What do protozoan and helminth pathogens have in common?
3) How does a multistage life cycle present a challenge for the immune system?
4) Give two examples of TLRs important for antiprotozoan defense and the PAMP/DAMP each recognizes.
5) How do antibodies combat protozoans?

Did You Get It? A Self-Test Quiz—Continued

6) Give four reasons why the Th1 response is crucial for antiprotozoan defense.
7) What CTL-mediated mechanism is most effective against protozoans and when?
8) What four types of leukocytes are involved in the Th2 response against helminth worms, and why are these cells important?
9) Outline three ways in which the contents of eosinophil granules combat helminths.
10) What three cytokines are important for antihelminth defense?
11) Give one example each of a disease caused by an ectoparasite itself and an ectoparasite acting as a vector.
12) Outline two ways each by which protozoans can avoid antibodies; phagosomes; complement.
13) Describe how protozoans and helminth worms interfere with T cell responses.

Section F

1) Can you define these terms? mycelium, hyphae, dimorphic, dermatophyte, mycoses, glucan, vomocytosis
2) Why is global warming a positive development for fungi but not humans?
3) Name two CLRs vital for antifungal defense and contrast their properties.
4) Describe four mechanisms of innate defense that combat fungi.
5) Describe the contribution of Th1, Th2 and Th17 cells to antifungal defense.
6) What are natural antibodies, and what do they recognize?
7) Fungal cells are lysed by complement. True or false?
8) Outline three ways in which the structure or products of fungi promote evasion of immune responses.
9) Give three ways by which a fungus can promote a less effective Th response; avoid humoral defense.

Section G

1) Can you define these terms? SE, vCJD, BSE, PrP^{res}, PrP^{c}
2) What is a prion, and how does it cause disease?
3) What is the connection between PrP^{c} and Alzheimer's disease?
4) Give two reasons why prions are poorly immunogenic.
5) Outline two pieces of scientific evidence suggesting that prions may induce innate immune responses.

Can You Extrapolate? Some Conceptual Questions

1) We noted in this chapter that a subset of B lymphocytes known as B-1 cells produces "natural antibodies" that are of the IgM isotype only and circulate in the periphery prior to antigen exposure. Keeping the idea of pattern recognition in mind, how might you argue that, despite being produced by B lymphocytes, these antibodies are actually part of the innate immune response to pathogens?
2) In many influenza epidemics and pandemics throughout history, those people who became most seriously ill or died were either the very young, the very old, or those who were immunocompromised in some way. In contrast, during the 2009 H1N1 influenza pandemic, the World Health Organization noted the following:
   a) Those who became seriously ill were often young adults in otherwise good health.
   b) Respiratory failure and shock were common causes of death.

   How might differences in immune responses to different influenza strains explain these observations?
3) If you were a nefarious individual attempting to create an intracellular pathogen that could prevent the most effective immune responses directed at it by the host, what mature cell type would you design the pathogen to infect and disable?

Would You Like To Read More?

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