Immunology

The Immune Response

As the immune system defends the host against pathogens, it uses different recognition systems To effectively eliminate the invading pathogen or its products. A response generated against a potential pathogen is called an immune response. The first line of defense, which is nonspecific to the invading pathogen, is rapidly mobilized at the initial site of infection but lacks immunologic memory and is called innate immunity. The second defense system is called adaptive immunity. It is specific for the pathogen and confers protective immunity to reinfection with that pathogen. Adaptive immunity can specifically recognize and destroy the pathogen because lymphocytes carry specialized cellular receptors and produce specific antibodies. A protein that is produced in response to a particular pathogen is called the antibody, and the substance that induces the production of antibodies is called the antigen. In summary, the innate immune response is effective and critical in eliminating most pathogens. However, if this initial mechanism fails; the adaptive immune response is induced that specifically confronts the pathogen and establishes immunity to that invading pathogen. Hence, both systems interact and collaborate to achieve the final goal of destroying the pathogen.

INNATE IMMUNITY

Innate immunity is an immediate response to a pathogen that does not confer long-lasting protective immunity. It is a nonspecific defense system and includes barriers to infectious agents, such as the skin (epithelium) and mucous membranes. It also includes many immune components important in the adaptive immune response, including phagocytic cells, natural killer (NK) cells, Toll-like receptors (TLRs), cytokines, and complement.

Barrier Functions of Innate Immunity

Few microorganisms can penetrate body surfaces. These surfaces have an epithelial cell layer as their barrier, which is present in the skin, airways, gastrointestinal (GI) tract, and genitourinary tract. The epithelial cell layer has tight junctions and produces a number of powerful antimicrobial peptides that help provide protection against invading pathogens. Lysozyme is an example of an antimicrobial peptide that dissolves some bacterial cell walls. Another major peptide of innate host defense with antimicrobial properties is defensin. Defensins are positively charged peptides located primarily in the GI and lower respiratory tracts that create holes in bacterial cell walls and hence disrupt the bacterial membrane. Neutrophils in the small intestine contain azurophilic granules that house the α-defensins that are released following TLR activation, whereas epithelial cells in the respiratory tract secrete a different defensin, called β defensin. The α-defensins have also been shown to possess antiviral activity. For example, α defensins can inhibit HIV (human immunodeficiency virus) binding to the CXCR4 (C-X-C chemokine receptor type 4) receptor and in this way interfere with virus entry into the cell.
The mucosal epithelium of the respiratory track offers another mode of protection from infection. Mucus, a complex mixture of mucins, proteins, proteases, and protease inhibitors, is a major component of the mucosal epithelium. Some bacteria attach to the surface epithelial cells by means of adhesive bacterial surface proteins (e.g., the pili of gonococci and Escherichia coli). However, the presence of mucus limits bacterial adhesion to these cell surfaces. Also, once Entrapped in the mucus, the bacteria are removed by ciliary clearance. thus, the mucosal surface and the ciliated epithelial cells tend to inhibit microbial adhesion and limit exposure time. Likewise, the GI track has mechanisms to inhibit bacteria. The acidity of the stomach and the proteolytic enzymes of the small intestine make this environment hostile to many bacteria.

An additional barrier to microbial invasion is the effect of the chemical environment. For example, the presence of an acidic pH in sweat and sebaceous secretions and, as mentioned previously, the low pH of the stomach has antimicrobial properties. Moreover, the production of fatty acids on the skin also tends to eliminate pathogenic organisms.

Mechanisms of Innate Immunity

Although innate immunity does not generate antigen specific protective immunity and does not rely on specific pathogen recognition, it nevertheless provides a powerful line of defense. In addition to the physiologic barriers of protection, the innate system has both cells and proteins (such as cytokines and complement) at its disposal. Phagocytic leukocytes, such as polymorphonuclear neutrophilic leukocytes (neutrophils), and macrophages along with NK cells are the primary cellular components to combat microbes. The interaction of the invading microbe with these cells and other cells throughout the body triggers the release of complement and numerous cytokines. Many of these cytokines are proinflammatory molecules such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and the interferons, and are induced through TLR interactions. Armed with these special tools, the host initiates its defense against the invading pathogen.

A. Microbial Sensors

When a pathogen enters the skin, it is confronted by macrophages and other phagocytic cells possessing "microbial sensors." There are three major groups of microbial sensors: (1) TLRs, (2) NOD-like receptors (NLRs), and (3) RIG-1–like helicases and MDA-5. The TLRs are the best studied of the microbial sensors. They are a family of evolutionary conserved pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs). They constitute a first line of defense against a variety of pathogens and play a critical role in initiating the innate immune response.

TLRs are type 1 transmembrane proteins with an extracellular domain, a single transmembrane α-helix, and a cytoplasmic domain. TLR recognition of these specific microbial patterns leads to a signal transduction cascade that generates a rapid and robust inflammatory response marked by cellular activation and cytokine release.

To date, 10 human TLRs have been identified, and each receptor appears to be involved in the recognition of a unique set of microbial patterns. For example, TLR2 recognizes various ligands (e.g., lipoteichoic acid) expressed by gram-positive bacteria, whereas TLR3 engages double stranded RNA (dsRNA) in viral replication. TLR1 and TLR6 recognize multiple diacyl peptides (e.g., mycoplasma), whereas TLR4 is specific for gram-negative lipopolysaccharides (LPS). TLR5, on the other hand, recognizes bacterial flagellin, and TLR7 and TLR8 interact with single-stranded RNA (ssRNA) in viral replication and TLR9 binds bacterial and viral DNA. At present, TLR10 remains an orphan receptor.

Another large family of innate receptors, NLRs, are located in the cytoplasm and serve as intracellular sensors for microbial products. They activate the nuclear factor kappa-light chain enhancer of activated B cells (NF-κB) pathway and drive inflammatory responses similar to the TLRs. The third group of microbial sensors is the RIG-1–like helicases and melanoma differentiation-associated protein 5 (MDA5). These are cytoplasmic sensors of viral ssRNA. The engagement of ssRNA with these sensors triggers the production of the type 1 IFNs. These IFNs are highly effective inhibitors of viral replication.

B. Cellular Components and Phagocytosis

The key elements of effective innate immunity are responses that are rapid, nonspecific, and of short duration. These features are the hallmark of the phagocytic process. During infection, circulating phagocytic cells increase and can participate in chemotaxis, migration, ingestion, and microbial killing. Any antigen (microorganism) that enters the body through the lymphatics, lung, or bloodstream is engulfed by phagocytic cells. Therefore, phagocytes present in the blood, lymphoid tissue, liver, spleen, lung, and other tissues are the cells responsible for the uptake and removal of foreign antigen.

Phagocytes include (1) monocytes and macrophages; (2) granulocytes, including neutrophils, eosinophils, and basophils; and (3) dendritic cells. Monocytes are small leukocytes that circulate in the blood and mature into macrophages that can be found in almost all tissues. For example, they are known as Kupffer cells in the liver and microglial cells in the nervous tissue. Macrophages are critical cells that engulf and kill pathogens, process and present antigen, and regulate immune reactivity by producing a variety of molecules (e.g., cytokines).

Granulocytes are leukocytes that contain densely staining granules. Neutrophils have a short half-life and are important phagocytic cells that destroy pathogens within intracellular vesicles. Eosinophils and basophils are less abundant and store granules containing enzymes and toxic proteins that can be released upon activation of the cells. Dendritic cells are also phagocytic and can degrade pathogens; however, their main role is to activate T cells in the adaptive immune response by acting as APCs and by producing regulatory cytokines (e.g., IFN-α).

Phagocytosis is a multistep process whereby a phagocytic cell, like a neutrophil, recognizes the pathogen, ingests it, and then destroys the engulfed organism. Once a pathogen enters the blood or tissue, the phagocytic cell migrates to that site. This migration is dependent on the release of chemoattractant signals produced by either the cells of the host or the pathogen. One chemoattractant is IL-8, a potent chemotactic cytokine that attracts neutrophils. More recently IL-17 has been shown to be an effective chemoattractant.

In the initial stage of the migration process, neutrophils attach to the endothelial cell surface by means of adhesion molecules, such as P-selectin. Neutrophils follow the chemokine attraction and migrate from the circulation through the endothelium into the tissues and to the site of infection. Here the neutrophil recognizes, engulfs, and internalizes the pathogen into an endocytic vesicle called a phagosome. Once inside the neutrophil, the pathogen is killed.

There are several antimicrobial mechanisms used by phagocytes to eliminate the pathogen. For example, (1) acidification occurs within the phagosome. The phagosome pH is 3.5–4.0, and this level of acidity is bacteriostatic or bactericidal. (2) Toxic oxygen-derived products are generated and include superoxide $O2^-$, hydrogen peroxide $H2O2$, and singlet oxygen $O2$. (3) Toxic nitrogen oxides are also produced, and nitric oxide NO is formed. (4) Phagocytic cells generate antimicrobial peptides that participate in pathogen killing.

In the macrophage, cathelicidin and macrophage elastase–derived peptides are found. The neutrophil, on the other hand, is rich in α-defensins, β-defensin, cathelicidin, and lactoferricin. All of these mechanisms are used by the phagocytes to destroy the pathogen. When the neutrophil completes its mission; it undergoes apoptosis and dies.

As already mentioned, phagocytosis can occur without antibody. However, phagocytosis is more efficient when antibodies are available to coat the surface of bacteria and facilitate their ingestion. This process is called opsonization and it can occur by the following mechanisms: (1) antibody alone can act as opsonin; (2) antibody and antigen can trigger the complement system (via the classic pathway) to generate opsonin; and (3) opsonin may be produced when the Alternative pathway is activated and C3 is generated. Macrophages have receptors on their membranes for the Fc portion of an antibody and for the complement component C3. Both of

these receptors facilitate the phagocytosis of the antibody coated pathogen.

C. Natural Killer Cells

Natural killer (NK) cells are large, granular lymphocytes morphologically related to T cells, which make up 10–15% of blood leukocytes. NK cells contribute to innate immunity by providing protection against viruses and other intracellular pathogens. NK cells have the ability to recognize and kill virus-infected cells and tumor cells.

NK cells express two types of surface receptors: (1) lectin-like NK-cell receptors that bind proteins not carbohydrates and (2) killer immunoglobulin-like receptors (KIRs) that recognize the major histocompatibility complex (MHC) class I molecules. These NK-cell receptors have both activation and inhibition properties. NK cells contain large amounts of granzyme and perforin, substances that mediate the cytotoxic actions of NK cells.

In addition, when antibody production is initiated in the adaptive immune response, NK cells play a critical role in antibody-dependent cellular cytotoxicity (ADCC). In this process, specific antibody binds to the target cell surface. The NK cell has Fc receptors that bind to the attached antibody and kill the cell. This property allows the NK cell another opportunity to inhibit the replication of viruses and intracellular bacteria.

NK cells and the IFN system are both integral parts of innate immunity that communicate with each other. NK cells are one of the two primary sources of IFN-γ, a potent antiviral and immunoregulating cytokine. Moreover, the lytic activity of NK cells is enhanced by the type 1 IFNs (IFN-α and IFN-β). These two cytokines are actually induced by the invading virus.

D. Complement System

The complement system is another key component of innate immunity. This system consists of 30 proteins found in the serum or on the membrane of selected cells that interact in a cascade. When complement is activated, it initiates a series of biochemical reactions that ultimately culminate in cellular lysis or destruction of the pathogen.

As described later in this chapter, there are three complement pathways: classic, alternative, and lectin. Even though each has a different initiating mechanism, they all result in the lysis of the offending invader. The alternative and lectin pathways serve as critical first lines of defense and provide immediate protection against microorganisms. The alternative complement pathway can be activated by microbial surfaces and it can proceed in the absence of antibody. Likewise, the lectin pathway also bypasses antibody and uses a lectin, mannose-binding lectin (MBL), to initiate events. The complement proteins can achieve their defense mission in several ways, including opsonization, lysis of bacteria, and amplification of inflammatory responses through the anaphylatoxins, C5a and C3a. Complement is described in more detail later in this chapter.

Some microbes have acquired mechanisms to sabotage the complement system and evade the immune response. For example, poxviruses, such as vaccinia virus and smallpox, encode a soluble protein with complement regulatory activity that leads to inhibition of the complement system.

E. Mediators of Inflammation and the Interferons

In the section on mechanisms of innate immunity, it was mentioned that various cells and complement components of innate immunity orchestrate their effects through the production of soluble mediators. These mediators include cytokines, prostaglandins, and leukotrienes. Here in this section, the role of these mediators in inflammation is outlined. A separate detailed description on cytokines is found in the section on adaptive immune response.

Injury to tissue initiates an inflammatory response. This response is dominated mainly by soluble mediators, referred to as cytokines. Cytokines may include inflammatory and anti-inflammatory cytokines, chemokines, adhesion molecules, and growth factors. During the innate immune response, leukocytes, such as macrophages, release a variety of cytokines, including IL-1 and TNF-α, and IL-6. The other mediators released from activated macrophages and other cells include prostaglandins and leukotrienes.

These inflammatory mediators regulate changes in local blood vessels. This begins with dilation of local arterioles and capillaries. During dilation, plasma escapes and accumulates in the area of injury. Fibrin is formed which occludes the lymphatic channels, limiting the spread of organisms.

A second effect of these mediators is to induce changes in the expression of adhesion molecules expressed on the surface of endothelial cells and leukocytes. Adhesion molecules (e.g., selectins and integrins) cause leukocytes to attach to the endothelial cells and thereby promote their movement across the vessel wall. Thus, cells stick to the capillary walls and then migrate out (extravasation) of the capillaries in the direction of the irritant.

This migration (chemotaxis) is stimulated by proteins in the inflammatory exudate, including some chemokines. A variety of cell types, including macrophages and endothelial cells, can produce chemokines. Once the phagocytic cells migrate to the site of infection, they can initiate the engulfment of microorganisms.

Fever is another common systemic manifestation of the inflammatory response and is a cardinal symptom of infectious disease. The main regulator of body temperature is the thermoregulatory center in the hypothalamus.

Among the substances capable of inducing fever (pyrogens) are endotoxins of gram-negative bacteria and cytokines (e.g., IL-1, IL-6, TNF-α, and the interferons) released from a variety of cells.

The interferons (IFNs) are critical cytokines that play a key role in defense against virus infections and other intracellular organisms, such as Toxoplasma gondii. Although the IFNs were first identified in 1957 as antiviral proteins, they are now recognized as critical immunoregulating proteins capable of altering various cellular processes, including cell growth, differentiation, gene transcription, and translation.

The IFN family consists of three groups. Type I IFNs comprise numerous genes and primarily include IFN-α and IFN-β. Type II IFN consists of a single gene that produces IFN-γ. IFN-λ is a third group of IFN-like cytokines that have more recently been described.

Virus infection itself triggers the production of type I IFNs. Following virus entry into a cell, the virus initiates replication and the viral nucleic acid interacts with specific microbial sensors (TLR3, TLR7, TLR9, RIG-1, and MDA-5). This interaction triggers cellular production of IFN that is secreted from the infected cell.

In contrast, the type II IFN, IFN-γ, is produced by activated NK cells in innate immune responses and by specifically sensitized T cells in adaptive immune responses. Moreover, the cytokines IL-2 and IL-12 can trigger T cells to produce IFN-γ.

The IFN system consists of a series of events leading to protection of a cell from virus replication. Once the IFN is produced by the infected cell or the activated NK cell or T cell, the IFN binds to its specific cellular receptor. The IFN receptor interaction activates the JAK, STAT signaling pathways. This process triggers activation of genes that initiate production of selected proteins that inhibit virus replication.

All of the IFNs share overlapping biological activities such as antiviral actions, antiproliferative actions, and immunoregulatory actions. However, they also have unique functions that are not

overlapping. For example, IFN-β is used successfully to treat patients with multiple sclerosis, whereas IFN-γ has been shown to exacerbate this disease.
These potent actions of the IFNs and the advances in biotechnology are the underlying factors that have identified the clinical relevance of the IFNs. In fact, many of the IFNs have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of infections, malignancies, autoimmunity, and immunodeficiency.

ADAPTIVE IMMUNITY

Unlike innate immunity, adaptive immunity is highly specific, has immunologic memory, and can respond rapidly and vigorously to a second antigen exposure. The adaptive immune response involves antibody-mediated and cell-mediated immune responses.

Cellular Basis of the Adaptive Immune Response

Lymphoid cells play a significant role in the adaptive immune response. During embryonic development, blood cell precursors (hematopoietic stem cells) originate in the fetal liver and other tissues; in postnatal life, the stem cells reside in the bone marrow. Stem cells may differentiate into cells of the myeloid or lymphoid series. The lymphoid progenitor cells develop into two main lymphocyte populations: B cells and T cells.
Stem cells destined to become B lymphocytes develop in the bone marrow. They rearrange their immunoglobulin genes and express a unique receptor for antigen on their cell surface. Following this step, they migrate to a secondary lymphoid organ (e.g., the spleen) and may be activated by an encounter with antigen to become antibody-secreting plasma cells.
T cells are lymphocytes that are produced in the bone marrow but travel to the thymus to mature. Here, they undergo variable diverse joining (VDJ) recombination of their β chain T cell receptor (TCR) DNA and their α chain TCR DNA. Once TCR rearrangement has occurred and positive and negative selection has terminated, these cells form T cell subclasses with specific functions (e.g., CD4 T cells, CD8 T cells). They are the source of cell-mediated immunity.
Figure 8-1 presents a summary of the specific immune processes that are reviewed in this section. The two arms of the immune response, cell-mediated and antibody-mediated, develop concurrently. In the antibody-mediated immune response, CD4 T lymphocytes recognize the pathogen's antigens bound to the class II MHC molecules on the surface of an antigen-presenting cell (APC) (e.g., macrophage, B cell), and as a consequence of this interaction, cytokines are produced that stimulate B cells to express antibodies that display specificity for the antigen. The B cells undergo clonal proliferation and differentiate into plasma cells.
In the cell-mediated immune response, the antigen–MHC class II complex is recognized by the CD4 T lymphocyte, whereas the antigen–MHC class I complex is recognized by CD8 T lymphocytes. Both subsets of T cells produce cytokines, become activated, and expand by clonal proliferation. The CD4 T cells that develop stimulate B cells to produce antibodies and promote delayed hypersensitivity while the CD8 T cells direct their activity mainly at the destruction of cells in tissue grafts, tumor cells, or virus-infected cells.

Antigens

An antigen is a substance that reacts with an antibody. Immunogens induce an immune response and most antigens are also immunogens. There are a wide variety of features that largely determine immunogenicity. They include the following:
(1) Recognition of foreignness: Generally, molecules recognized as "self" are not immunogenic. To be immunogenic, molecules must be recognized as foreign ("nonself").

(2) Size: The most potent immunogens are usually large, complex proteins. Molecules with a molecular weight less than 10,000 are weakly immunogenic, and as expected very small molecules are nonimmunogenic. Some small molecules, called haptens, become immunogenic only when linked to a carrier protein. An example is seen with lipids and amino acids that are nonimmunogenic haptens. They require conjunction with a carrier protein or polysaccharide before they can be immunogenic or generate an immune response.
(3) Chemical and structural complexity: Chemical complexity is another key feature of immunogenicity. For example, amino acid homopolymers are less immunogenic than heteropolymers that contain two or more different amino acids.
(4) Genetic constitution of the host: Because of differences in MHC alleles, two strains of the same species of animal may respond differently to the same antigen.
(5) Dosage, route, and timing of antigen administration: Other factors that affect immunogenicity include concentration of antigen administered, route of administration, and timing of antigen administration.
These concepts of immunogenicity are important for designing vaccines in which enhancing immunogenicity is key. However, methods to reduce immunogenicity are also a consideration in protein drug design. This can be seen in an individual who may respond to a certain drug and produce anti-drug antibodies. These anti-drug antibodies may inhibit drug efficacy.
Finally, it should be noted that it is possible to enhance the immunogenicity of a substance by combining it with an adjuvant. Adjuvants are substances that stimulate the immune response by facilitating uptake into APCs.

Antigen Recognition Molecules
During the immune response a recognition system capable of distinguishing self from nonself is essential for effective immunity. This section of the chapter concentrates on the molecules used to recognize foreign antigens. Molecules of the MHC and antigen presentation are reviewed first, followed by an overview of the structure and function of antibodies and lastly, an outline of the specific receptors for antigen recognition (i.e., the B-cell receptor [BCR] and the TCR for antigen) is presented.

The Major Histocompatibility Complex
Historically, the major histocompatibility complex (MHC) was first discovered as a genetic locus that encoded a group of antigens responsible for the rejection of tumor grafts. It is now known that the gene products of this region are the major antigens recognized in transplantation rejection. It is also clear that the MHC molecules bind peptide antigens and present them to T cells. Hence, these molecules are responsible for T-cell antigen recognition and play a significant role in controlling a variety of basic immunologic functions.
It should also be noted that the TCR is different from antibody. Antibody molecules bind antigen directly, whereas the TCR only recognizes peptide antigens presented in the context of the MHC molecule on the APC. The TCR is specific for antigen, but the antigen must be presented on a self-MHC molecule. The TCR is also specific for the MHC molecule. Should this antigen be presented by another allelic form of the MHC molecule in vitro, the TCR does not recognize the complex. This is known as MHC restriction.
The MHC is a cluster of well-studied genes closely associated in humans on chromosome 6. The human MHC is called the human leukocyte antigen (HLA) complex. Among the many important genes in the human MHC are those that encode the classes I, II, and III MHC proteins.
As outlined in Table 8-1, MHC class I proteins are encoded by the HLA-A, -B, and -C genes. These proteins are made up of two chains: (1) a transmembrane glycoprotein of MW 45,000,

noncovalently associated with (2) a non–MHC-encoded polypeptide of MW 12,000 that is known as β2-microglobulin.
MHC class I molecules are expressed on nearly all nucleated cells in the body. Key exceptions are observed on cells in the retina and brain.
Class II proteins are encoded by the HLA-D region. The MHC class II proteins consist of three main families: the HLA-DP–, DQ-, and DR-encoded molecules This locus controls immune responsiveness and different allelic forms of these genes confer differences in the ability of an individual to mount an immune response.
The HLA-D locus-encoded molecules are cell surface heterodimers that contain two subunits designated α and β that have molecular weights of approximately 33,000 and 29,000 Da, respectively. Unlike class I proteins, the MHC class II proteins have a rather restricted tissue distribution and are constitutively expressed on macrophages, dendritic cells, and B cells. However, the expression of these molecules on other cell types (e.g., endothelial cells or epithelial cells) requires induction by IFN-γ.
The MHC class I locus also contains genes that encode proteins required in antigen processing (e.g., transporters associated with antigen processing [TAPs]). MHC class III locus encodes complement proteins and several cytokines.
The MHC classes I and II genes exhibit extraordinary genetic variability. Genetic mapping studies showed that there is a high degree of polymorphism in the MHC and different individuals generally express different MHC allelic variants (MHC restriction). It has been noted that over 300 different allelic variants have been defined at some HLA loci. Currently, the MHC genes are the most polymorphic genes known. Each individual inherits a restricted set of alleles from his or her parent. A cluster of tightly linked MHC genes are inherited as a block or haplotype.
In 1987, the three-dimensional structure of the MHC classes I and II proteins was revealed using x-ray crystallography. This elegant work provided critical information on how the MHC proteins function and trigger the immune response. X-ray analysis (Figure 8-3) demonstrates that the entire structure looks like a cleft whose sides are formed by the α helices and whose floor is shaped by the β-pleated sheets. The x-ray analysis also shows that the cleft is occupied by a peptide. In essence, the TCR sees the peptide antigen bound in a cleft provided by the MHC protein.
The MHC proteins display broad specificity for peptide antigens. In fact, many different peptides can be presented by a different MHC allele. A key to this model is that the MHC polymorphism allows for the binding of many specific and different peptides in the cleft. This means that different alleles can bind and present different peptide antigens.

Antigen Processing and Presentation
Antigen processing and presentation represent the hallmark of the adaptive immune response. This complex mechanism of antigen recognition begins with antigens that become associated with self-MHC molecules for presentation to T cells with appropriate receptors. Proteins from exogenous antigens, such as bacteria, are internalized by the APC (dendritic cells or macrophages) and undergo denaturation or partial proteolysis in the endocytic vesicles within the APC. While in the endosomal compartment, these peptide fragments fuse with exocytic vesicles containing MHC class II molecules. As noted in Figure 8-2, this step exposes the appropriate linear peptide fragment that eventually becomes expressed on the surface of the APC (as the peptide-MHC complex).
The MHC class II molecules are synthesized in the rough endoplasmic reticulum (ER) and then they proceed out through the Golgi apparatus. The invariant chain, a polypeptide that helps

transport the MHC molecules, complexes with the MHC class II complex in an endosome. This vesicle is called the MHC class II compartment. This invariant chain is useful and blocks the binding of self-endogenous cellular peptides into the MHC class II complex. The invariant chain is now enzymatically removed. Through a series of steps, the MHC class II binds exogenous antigen (peptide fragments) and is transported to the cell membrane for presentation.
In brief, cytosolic proteins are broken down by a proteolytic complex called the proteasome. The cytosolic peptides gain access to nascent MHC class I molecules in the rough ER via the peptide transporter systems (TAPs). The TAP genes are also encoded in the MHC. Within the lumen of the ER, peptide antigens approximately 8–10 residues in length complex with nascent MHC class I proteins and cooperate with β2-microglobulin to create a stable, fully folded MHC class I–peptide antigen complex that is then transported to the cell surface for display and recognition by CD8 cytotoxic T cells. The binding groove of the class I molecule is more constrained than that of the class II molecule, and therefore, shorter peptides are found in class I than in class II MHC molecules. Once the cytotoxic T cell recognizes the MHC class I peptide antigen, it can now kill the virus-infected cell.
Several viruses attempt to defeat the immune response by interfering with the antigen-processing pathways. For example, an HIV Tat protein is able to inhibit expression of class I MHC molecules. A herpesvirus protein binds to the TAPs, preventing transport of viral peptides into the ER, where class I molecules are being synthesized. A consequence of these inhibitory mechanisms is that the infected cells are not recognized by cytotoxic lymphocytes.
Some bacterial and viral antigens are able to activate large numbers of T cells through a special pathway. These proteins are called superantigens. Superantigens do not require processing and therefore are able to bind to MHC molecules outside the peptide-binding cleft (Figure 8-4B). Compared to the standard antigen-induced T-cell response where a small number of T cells are activated, superantigens can stimulate much larger numbers (~25% more) of the T cells. Classic examples of superantigens include certain bacterial toxins, including the staphylococcal enterotoxins, toxic shock syndrome toxin, and group A streptococcal pyrogenic exotoxin A. A consequence of this massive activation of T cells is the overproduction of cytokines, in particular, IFN-γ. IFN-γ in turn activates macrophages to produce IL-1, IL-6, and TNF-α, all which may contribute to a “cytokine storm” causing severe symptoms of shock and multiple organ failure.

B Cells and Antibodies
Humoral immunity is mediated by antibodies. Each individual has a large pool of unique B lymphocytes (~10^11) that have a life span of days or weeks and are found in the blood, lymph, bone marrow, lymph nodes, and gut-associated lymphoid tissues (eg, tonsils, Peyer patches, appendix).

A. B Cell Receptor for Antigen
B cells display a single homogenous clonal immunoglobulin molecule (~10^5 copies/cell) on their surface. These immunoglobulins serve as receptors (B-cell receptors [BCRs]) for a specific antigen, so that each B cell can respond to only one antigen or a closely related group of antigens. All immature B cells carry IgM immunoglobulin on their surface, and most also express IgD. Additionally, B cells have surface receptors for the Fc portion of immunoglobulins as well as for several complement components.
An antigen interacts with the B lymphocyte that shows the best “fit” by virtue of its immunoglobulin surface receptor. When antigen binds to this BCR, the B cell is stimulated to divide and form a clone (clonal selection). Such selected B cells proliferate and differentiate to

become plasma cells that secrete antibody. Because each person can make approximately 10^11 different antibody molecules, there is an antigen-binding site on a B cell to fit almost any antigenic determinant.
The initial step in antibody formation begins with the binding of antigen to the surface immunoglobulin via the BCR. Then the following steps ensue: (1) The BCR with its bound antigen is internalized by the B cell and the antigen is degraded to yield peptides that are then returned to the cell surface bound to MHC class II molecules. (2) This MHC class II–peptide complex on B cells is recognized by antigen-specific helper (CD4) T cells. These T cells have already interacted with antigen-presenting dendritic cells and have differentiated in response to the same pathogen. This interaction can occur because the B cell and the T cell that have encountered antigen migrate toward the boundaries between B- and T-cell areas in the secondary lymphoid tissue. (3) Chemokines, such as CXCL13 and its receptor, CXCR5, play an important role in this migration process. (4) The CD40 ligand on T cells binds to CD40 on B cells, and the T cell produces IL-4, IL-5, and IL-6, which induce B-cell proliferation. (5) Finally, the activated B cells migrate into follicles and proliferate to form germinal centers; here somatic hypermutation and immunoglobulin class switching occur. Germinal center B cells that survive this process now differentiate into either antibody-producing plasma cells or memory B cells. Additional details on this topic can be found in the chapter reference, Murphy et al (2012).
It should be noted that some bacterial antigens can directly stimulate this antibody production and do not require T cell help to activate B cells. These antigens are usually bacterial polysaccharides and LPS. These thymus T-cell–independent antigens induce B-cell responses with limited class switching and do not induce memory B cells. By passing T-cell participation can be an advantage for the host because an expedited immune response (IgM production) can be generated against selected organisms, such as, Haemophilus influenzae and Streptococcus pneumoniae.

B. Antibody Structure and Function

Antibodies are immunoglobulins, which react specifically with the antigen that stimulated their production. They make up about 20% of the plasma proteins. Antibodies generated in response to a single complex antigen are heterogeneous because they are formed by many different clones of cells. Each clone expresses an antibody capable of reacting with a different antigenic determinant on the complex antigen. These antibodies are called polyclonal. In contrast, immunoglobulins that arise from a single clone of cells, such as a plasma cell tumor (myeloma), are homogeneous and are called monoclonal antibodies. Monoclonal antibodies can be produced in vitro by fusing a myeloma cell with an antibody-producing B lymphocyte.
The immunoglobulin (Ig) molecules share common structural features; that is, all the Ig molecules are composed of light and heavy polypeptide chains. The terms light and heavy refer to their molecular weight. The light chains have a molecular weight of approximately 25,000, whereas the heavy chains have a molecular weight of approximately 50,000. Each Ig molecule consists of two identical light (L) chains and two identical heavy (H) chains linked by disulfide bridges.
The L chains can be either κ (kappa) or λ (lambda) and their classification is made based on the amino acid differences in their constant regions. Both light chain types can occur in all classes of immunoglobulins (IgG, IgM, IgA, IgD, and IgE), but any one Ig molecule contains only one type of L chain. The amino terminal portion of each L chain contains part of the antigen-binding site.
Similarly, the H chains are distinct for each of the five immunoglobulin classes and are designated γ (gamma), μ (mu), α (alpha), δ (delta), and ε (epsilon). The amino terminal portion

of each H chain participates in the antigen-binding site; the other (carboxyl) terminal forms the Fc fragment. The Fc portion of the Ig molecule participates in various biologic activities such as complement activation. Therefore, an individual antibody molecule consists of identical H chains and identical L chains. The simplest antibody molecule has a Y shape and consists of four polypeptide chains: two H chains and two L chains. The four chains are covalently linked by disulfide bonds.

When studying the Ig molecule structure, it was identified experimentally that an antibody molecule, such as IgG, can be split into two fragments by the proteolytic enzyme papain. When this happens, the peptide bonds in the hinge region are broken. The antigen-binding activity is associated with one of these fragments, the Fab portion. The second fragment is the Fc portion that is involved in placental transfer, complement fixation, attachment to various cells, and other biologic activities.

The L and H chains of an Ig molecule are subdivided into variable regions and constant regions. The regions are composed of three-dimensionally folded, repeating segments called domains. By using high-resolution x-ray crystallography, the structure of these domains has been determined. An L chain is composed of one variable domain (VL) and one constant domain (CL) whereas most H chains have one variable domain (VH) and three or more constant domains (CH). Each domain is approximately 110 amino acids in length. The variable regions of the Ig molecule are involved in antigen binding, whereas the constant regions are responsible for the biologic functions described later in this section.

Within the variable regions of both the L and H chains are subregions consisting of extremely variable amino acid sequences, called hypervariable, that cooperate in space to form the antigen-binding site. The hypervariable regions form the area of the Ig molecule complementary in structure to the antigenic determinant (epitope). This area is known as the complementarity-determining region (CDR). Only 5–10 amino acids in each hypervariable region constitute the antigen-binding site. Antigen binding is noncovalent, involving van der Waals and electrostatic as well as other weak forces.

Immunoglobulin Classes

A. IgG

IgG is the major class of immunoglobulin present in the serum. The IgG molecule consists of two L chains and two H chains (H2L2). There are four subclasses of IgG: IgG1, IgG2, IgG3, and IgG4. Each subtype contains a distinct but related H chain and each differs somewhat regarding their biological activities.

IgG1 represents 65% of the total IgG. IgG2 is directed against polysaccharide antigens and may be an important host defense against encapsulated bacteria. IgG3 is an effective activator of complement due to its rigid hinge region, whereas IgG4 does not activate complement due to its compact structure.

IgG is the only immunoglobulin class to cross the placenta and therefore is the most abundant immunoglobulin in newborns. Isotype-specific transport of IgG across the placenta occurs with preference for IgG1 and IgG3 subclasses. IgG also mediates opsonization of antigen through binding of antigen-antibody complexes to Fc receptors on macrophages and other cells.

B. IgM

The first immunoglobulin produced in response to an antigen is IgM. IgM is secreted as a pentamer and is composed of five H2L2 units (similar to one IgG unit) and one molecule of a J chain. The pentamer (MW 900,000) has a total of 10 identical antigen-binding sites and thus a valence of 10.

It is the most efficient immunoglobulin in agglutination, complement fixation, and other antigen–antibody reactions and is important also in defense against bacteria and viruses. Because its interaction with antigen can involve all 10 binding sites, it has the highest binding capacity and cross-linking of all the immunoglobulins.
Evaluating the presence of serum IgM may be useful in the diagnosis of certain infectious diseases. For example, IgM does not cross the placenta and the presence of the IgM antibody in the fetus or newborn provides evidence of intrauterine infection.

C. IgA
IgA is the major immunoglobulin responsible for mucosal immunity. The levels of IgA in the serum are low, consisting of only 10–15% of total serum immunoglobulins present. In contrast, IgA is the predominate class of immunoglobulin found in extravascular secretions. Thus, plasma cells located in glands and mucous membranes mainly produce IgA.
Therefore, IgA is found in secretions such as milk, saliva, and tears, and in other secretions of the respiratory, intestinal, and genital tracts. These locations place IgA in contact with the external environment and therefore can be the first line of defense against bacteria and viruses.
The properties of the IgA molecule are different depending on where IgA is located. In serum, IgA is secreted as a monomer resembling IgG. In mucous secretions, IgA is a dimer and is referred to as secretory IgA. This secretory IgA consists of two monomers that contain two additional polypeptides: the J chain that stabilizes the molecule and a secretory component that is incorporated into the secretory IgA when it is transported through an epithelial cell.
There are at least two IgA subclasses: IgA1 and IgA2. Some bacteria (e.g., Neisseria spp.) can destroy IgA1 by producing a protease and can thus overcome antibody-mediated resistance on mucosal surfaces.

D. IgE
The IgE immunoglobulin is present in very low quantities in the serum. The Fc region of IgE binds to its high-affinity receptor on the surface of mast cells, basophils, and eosinophils. This bound IgE acts as a receptor for the specific antigen that stimulated its production and the resulting antigen–antibody complex triggers allergic responses of the immediate (anaphylactic) type through the release of inflammatory mediators such as histamine.

E. IgD
Serum IgD is present only in trace amounts. However, IgD is the major surface bound immunoglobulin on mature B lymphocytes that have not yet encountered antigen. These B cells contain IgD and IgM at a ratio of 3 to 1. At the present time, the function of IgD is unclear.

Antibody Responses
A. The Primary Response
When an individual encounters an antigen for the first time, the antibody produced in response to that antigen is detectable in the serum within days or weeks. This time can vary depending on the nature and dose of the antigen and the route of administration (e.g., oral, parenteral). The serum antibody concentration continues to rise for several weeks and then declines; the antibody level may drop to very low levels. The first antibodies produced are IgM. Then, IgG, IgA, or both Ig are made. IgM levels tend to decline sooner than IgG levels.

B. The Secondary Response

In the event of a second encounter with the same antigen months or years after the primary response, the second antibody response is more rapid and generates higher levels than during the primary response. This change in response is attributed to the persistence of antigen-sensitive memory cells that were generated during the primary immune response. In the secondary response, the amount of IgM produced is qualitatively similar to that produced after the first contact with the antigen; however, more IgG is produced, and the level of IgG tends to persist much longer than that produced in the primary response. Furthermore, such antibody tends to bind antigen more firmly (with higher affinity) and thus to dissociate less easily.

Protective Functions of Antibodies

The protective role of antibodies is based on the fact that specific antibodies are generated that recognize and bind to specific pathogens. This interaction triggers a series of host defense responses. Antibodies can produce resistance to infection by five major mechanisms:

1. Enhanced phagocytosis—Antibodies produce resistance by opsonizing (coating) organisms, which make them more readily ingested by phagocytes. In addition, antibody-mediated immunity against the pathogen is most effective when directed against microbial infections in which virulence is related to polysaccharide capsules (e.g., pneumococcus, Haemophilus spp., Neisseria spp.). In these infections, antibodies complex with the capsular antigens and make the organisms susceptible to ingestion by phagocytic cells. This engulfment leads to pathogen destruction.
2. Virus neutralization—Antibodies directed against specific viral proteins can bind to the virus and block the ability of the virus particle to attach to its cellular receptor. Because the virus can no longer invade the cell, it cannot replicate.
3. Neutralization of toxins—Antibodies can neutralize toxins of microorganisms (e.g., diphtheria, tetanus, and botulism) and inactivate their harmful effects.
4. Complement-mediated lysis—The attachment of antibodies to viral proteins on virus-infected cells, tumor cells, or to a microbial cell wall can activate the complement system leading to cell lysis.
5. Antibody-dependent cell cytotoxicity (ADCC)—The attachment of virus-specific antibodies to viral proteins on a virus-infected cell can lead to the lytic destruction of the infected cell. This lysis is mediated by a killer cell (NK, macrophage, neutrophil) that binds to the Fc portion of that bound antibody. ADCC by eosinophils is an important defense mechanism against helminths. IgE coats the worms and eosinophils attach to the Fc portion of IgE triggering eosinophil degranulation.

Forms of Immunity

Because antibodies are protective, strategies have been developed to induce their production (active immunity) or to administer preformed antibodies to the host (passive immunity).

A. Active Immunity

Active immunity is conferred when an individual comes in contact with a foreign antigen (infectious agent). This immunity can occur in the setting of a clinical or subclinical infection, immunization with live or killed organism, exposure to microbial products (e.g., toxins, toxoids), or transplantation of foreign tissue. In all these cases the individual actively produces antibodies. The antibody produced during active immunity is long lasting. However, protection is delayed until antibody production reaches an effective level.

B. Passive Immunity

Passive immunity is generated by the administration of preformed antibodies. The main advantage of passive immunization is that the recipient receives a large concentration of antibody immediately. This does not confer long-term protection but is useful when the patient has no time to produce an antibody response. Passive immunity is helpful against certain viruses (e.g., hepatitis B virus) after a needle-stick injury to someone who has not been vaccinated or in cases of immune deficiency where antibody cannot be produced.

In addition to the antibody-mediated protective effects, harmful effects from antibody administration can also be seen. In passive immunity it is possible to initiate hypersensitivity reactions if the antibody is from another species. However, in active immunity, the binding of antibodies to the antigen leads to the formation of circulating immune complexes. The deposition of these complexes may be an important feature in the development of organ dysfunction. For example, immune complexes may deposit in the kidney and induce glomerulonephritis, which can result following streptococcal infections.

T Cells

A. Cell-Mediated Immunity

Within the adaptive immune response, the cooperative interaction of both antibody- and cell-mediated immunity provides the best opportunity for combating infection. In fact, effective antibody responses depend on the activation of T cells. This section directs attention to T-cell activation, T cell recognition of antigen, and T cell subsets and their function as well as T cell development, proliferation, and differentiation.

1. Development of T cells—As previously mentioned, T cells are derived from the same hematopoietic stem cells as are the B cells. Within the thymus, T cells mature and undergo differentiation. Under the influence of thymic hormones, T cells differentiate into committed cells expressing a specific TCR. These T cells have undergone VDJ recombination of their β chain and then rearrangement of their α chains.

These T cells now undergo two processes: one positive and one negative. During positive selection, cells that recognize self-peptide plus self-MHC with weak affinity will survive. These cells are now termed self-MHC restricted. During negative selection, the cells that recognize self-peptide plus self-MHC with high affinity are killed. These survivor cells, CD4+ CD8+ double positive T cells, continue to mature into either CD4+ or CD8+ T cells. Only a minority of developing T cells express the appropriate receptors to be retained and enter the periphery where they join the mature T-cell pool.

2. T cell receptor for antigen—The TCR is the recognition molecule for T cells. The TCR is a transmembrane heterodimeric protein containing two disulfide-linked chains. It is composed of two different classes of TCR called: alpha-beta (α and β) and gamma-delta (γ and δ). The majority of the T cells contain the αβ TCR phenotype. However, a smaller percentage of T cells express the γδ TCR. The αβ T cells are subdivided by their surface markers: CD4 or CD8. Little is known about the activities of the γδ T cells. The γδ T cells are primarily located in the epithelial linings of the reproductive and GI tracts.

The structure of the TCR resembles the Fab fragment of an immunoglobulin molecule; that is, the TCR has both variable and constant regions. More specifically, each chain has two extracellular domains: a variable region and a constant region. The constant region is closest to the cell membrane whereas the variable region binds the peptide-MHC complex. When the TCR engages the antigen peptide-MHC complex, a series of biochemical events occur.

As outlined for the immunoglobulins, the diversity of the TCR is similar to that described for the BCR. The α chain of the TCR is the result of VJ recombination whereas the β chain is generated

by VDJ recombination. These segments can combine randomly in different ways to generate the complex TCR.

The TCR complex is formed by the highly variable α and β chains of the TCR plus the invariant CD3 proteins. The invariant proteins of the CD3 complex are responsible for transducing the signal received by the TCR when antigen recognition occurs. The different proteins of the CD3 complex are transmembrane proteins that can interact with cytosolic tyrosine kinases that initiate signal transduction leading to gene transcription, cell activation, and initiation of the functional activities of T cells.

In addition to the TCR complex, the T cell signal is also enhanced by the presence of coreceptors (second signal). The CD4 and CD8 molecules on the T cell membrane function as coreceptor molecules. During recognition of antigen, the CD4 and CD8 molecules interact with the TCR complex and with MHC molecules on the APC. CD4 binds to MHC class II molecules and CD8 binds to MHC class I molecules.

3. T cell proliferation and differentiation—T cell proliferation depends on a series of events. In MHC class II presentation two signals are required for the naïve CD4 T cell activation to occur. The first signal comes from the TCR on the T cell interacting with an MHC-peptide complex presented on the APC. The CD4 glycoprotein on the naïve T cell acts as a coreceptor, binding to MHC class II molecules. This binding event helps ensure stability between the T cell and the APC.

The second signal (co-stimulation) that is required for T cell activation is derived from the interaction of the B7 family costimulatory molecules (B7-1/B7-2 also identified as CD80 and CD86) on the APC with CD28 on the T cell. These are the major costimulatory molecules. Upon completion of these two stimulation steps (e.g., TCR binding to MHC class II–peptide complex and CD28 binding to B7-1/B7-2), a set of biochemical pathways are triggered in the cell that results in DNA synthesis and proliferation.

During these events, the T cell secretes cytokines, mainly IL-2 and IFN-γ, and increases the expression of IL-2 receptors. These T cells are able to proliferate and differentiate into effector cells.

CD8 T-cell activation occurs when the TCR interacts with the MHC class I–peptide complex on the infected cell. The CD8 glycoprotein on the T cell acts as a coreceptor, binding to MHC class I molecule on the APC. Again, this interaction keeps the two cells bound together during antigen-specific activation. Once activated, the cytotoxic T cell produces IL-2 and IFN-γ, growth and differentiation factors for T cells. Unlike CD4 cell activation, CD8 T cell activation in most cases is independent of co-stimulation, and the virus-infected cell is destroyed through cytotoxic granules released from the CD8 T cell.

B. T Cell Effector Functions

1. CD4 effector cells—Proliferating CD4 T cells can become one of four main categories of effector T cells: Th1 cells, Th2 cells, Th17 cells, or T regulatory (T reg) cells.

Th1—Th1 cells are triggered by IL-2 and IL-12 and either activate macrophages or cause B cells to switch to produce different subclasses of IgG. In either case, this can promote bacterial clearance either by direct destruction in the IFN-γ–activated macrophage or by destruction after phagocytosis of opsonized particles. These Th1 cells also produce IL-2 and IFN-γ.

Th2—In an environment where IL-4 is being produced, Th2 cells predominate and activate mast cells and eosinophils, and cause B cells to synthesize IgE. This aids in the response to helminths. The Th2 cells secrete IL-4, IL-5, IL-9, and IL-13.

Th17—When TGF-β, IL-6 and IL-23 are present CD4 T cells can become Th17 cells. These cells produce IL-17, IL-21 and IL-22. IL-17 is a cytokine that induces stromal and epithelial

cells to produce IL-8. IL-8 is a potent chemokine that is responsible for the recruitment of neutrophils and macrophages to infected tissues.

T regs—CD4 T cells can become T regulatory (T regs) when they are exposed to TGF-β alone. T reg cells function by suppressing T cell responses. They are identified by expression of CD4 and CD25 molecules on their surface and the transcription factor, Foxp3. T reg cells produce TGF-β and IL-10, which can suppress immune responses.

2. CD8 effector cells—CD8 cells differentiate into effector cytotoxic cells by engagement of their TCR and recognition of class I MHC–peptide complex on the surface on an infected cell. Following recognition, the CD8 T cell proceeds to kill the infected cell. The primary method of killing is through cytotoxic granules containing perforin, the family of granzymes, and a third protein recently identified, granulysin. The CD8 T cell releases perforin that helps granzyme and granulysin enter the infected cell. Granzyme initiates apoptosis (programmed cell death) by activating cellular caspases.

It should be noted that a similar process occurs with CD8 T cell recognition of tumor cells.

COMPLEMENT

The complement system, a complex and sophisticated cascade of various proteins, is designed to provide defense against microbial invaders. The complement system includes serum and membrane-bound proteins that participate in both innate and adaptive immunity. These proteins are highly regulated and interact via a series of proteolytic cascades. Many of the complement components are proenzymes, which must be cleaved to form active enzymes.

Biologic Effects of Complement

Activated complement proteins initiate a variety of functions that result in four major outcomes: (1) cytolysis, (2) chemotaxis, (3) opsonization, and (4) anaphylatoxins.

1. Cytolysis is the lysis of cells, such as bacteria, virus-infected cells, and tumor cells. This process occurs through the development of the membrane attack complex (MAC) (C5b, 6, 7, 8, 9), which inserts into the membrane of an organism or cell. The MAC creates holes in the cell membrane, which leads to loss of osmotic integrity and rupture of the microbe or cell.
2. Chemotaxis is the directed movement of leukocytes up a gradient concentration toward the site of infection. This movement is in response to a chemotactic factor. One of the most important chemotactic substances is C5a, a fragment of C5 that stimulates movement of neutrophils and monocytes to sites of inflammation.
3. Opsonization is a term used to describe how antibodies or C3b can enhance phagocytic engulfment of microbes. Macrophages and neutrophils have receptors for C3b and therefore can bind C3b-coated organisms. This binding trigger phagocytosis.
4. Anaphylatoxins promote vasodilation and increase vascular permeability. Two complement components, C3a and C5a, are potent anaphylatoxins. Both bind to receptors on mast cells and basophils triggering them to release histamine. This event results in an increased blood flow to the site of infection, allowing more complement, antibodies, and immune cells to enter the site of infection.

Complement Pathways

There are three major pathways that activate complement: the classic pathway, alternative pathway, and MBL pathway. Each of these pathways results in the formation of the MAC. All three pathways lead to the release of C5 convertase, which breaks down C5 into C5a and C5b. As mentioned previously, C5a is an anaphylatoxin as well as a chemotactic factor. C5b binds to C6 and C7 to form a complex that inserts into the membrane bilayer. C8 then binds to the C5b–C6–C7 complex followed by polymerization of up to sixteen C9 molecules to produce the MAC.

The MAC now generates a pore in the membrane and causes cytolysis by allowing free passage of water across the cell membrane.

The Classic Pathway

C1, which binds to the Fc region of an immunoglobulin, is composed of three proteins: C1q, C1r, and C1s. C1q is a complex is now exposed and ready for C1 to attach. The classic pathway is now activated and the infected cell is destroyed by the MAC.

CYTOKINES

Over the last two decades, we have witnessed an explosion in cytokine biology. Cytokines are potent, low-molecular-weight protein cell regulators produced transiently and locally by numerous cell types. Today we recognize that cytokines are multifunctional proteins whose biological properties suggest a key role in hematopoiesis, immunity, infectious disease, tumorigenesis, homeostasis, tissue repair, and cellular development and growth.

Cytokines usually act as signaling molecules by binding to their own glycoprotein receptors on cell membranes. This initial interaction is followed by a relay of the signal to the cell nucleus. Signal transduction is mediated as in many hormone-receptor systems via kinase-mediated phosphorylation of cytoplasmic proteins. In fact, tyrosine kinase activity is intrinsic to many cytokine receptors. Because of their role in multiple immunologic activities, cytokines are mentioned throughout this chapter.

Classification and Functions

Cytokines can be categorized into groups based on their common functions. Examples of functional categories include immunoregulatory, proinflammatory, anti-inflammatory, chemokines, adhesion molecules, growth and differentiation. Because of its major role in antigen presentation, an important immunoregulatory cytokine is IFN-γ. Proinflammatory cytokines are commonly seen in infectious diseases, and they include IL-1, IL-6, TNF-α, and the IFNs. The anti-inflammatory cytokines include TGF-β, IL-10, IL-11, and IFN-β. These may be required to dampen or downregulate an overactive inflammatory response. Cytokines that have a key role in growth and differentiation include the colony-stimulating factors (CSFs) and stem cell factor.

Cytokines in Immune Cell Development and Host Defense to Infections

Naïve CD4+ T cells can differentiate into different lineages depending on the exogenous cytokine environment. Th1 cells develop in the presence of IL-12; Th2 cells develop in the presence of IL-4; and Th17 cells develop in the presence of TGF-β, IL-6, and IL-23, and T reg cells are formed in the presence of TGF-β alone. Each of these four T-cell lineages produce cytokines that play a pivotal role in host defense against selective microorganisms.

Th1 cells produce IL-2 and IFN-γ, cytokines that can effectively control virus infections and intracellular organisms such as mycobacteria and Toxoplasma gondii. IFN-γ is a key activator of macrophages and cytotoxic CD8+ T cells. Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13, cytokines that drive IgE responses and help control parasitic infections.

Th17 cells produce IL-17, a cytokine that attracts neutrophils and plays protective host defense roles at the epithelial and mucosal barriers. IL-17 has been shown to limit infections in the skin against Staphylococcus aureus, in the colon against Citrobacter rodentium, in the lung against Klebsiella pneumoniae, in the mouth against Candida albicans and in the vagina against Chlamydia. IL-17 has also been shown to inhibit fungal infections caused by Pneumocystis carinii. Recent studies have shown that mutations in IL-17 and IL-17 receptor genes predispose individuals to chronic muco-candidiasis caused by C albicans.

Finally, T regs are regulatory T cells that help suppress T-cell proliferation and maintain tolerance to self-antigens. It has been suggested that T regs functions are facilitated, in part, by the production of immunosuppressive cytokines, IL-10 and TGF-β.
This analysis of T cell differentiation demonstrates how T cell subsets secrete their own set of cytokines that have distinct regulatory properties. Thus, cytokines orchestrate the type of protective immune response that is generated.

Clinical Applications
Today there are at least four major clinical applications of cytokines. First, cytokines can serve as biomarkers of disease and provide clues for mechanisms of disease. For example, the proinflammatory cytokines TNF-α, IL-1, and IL-6 can be detected in the sera of patients with septic shock. These cytokines appear to play a critical role in the development of septic shock, and tracking their presence may be of prognostic value in severe sepsis.
Second, the measurement of cytokine production in vitro is a useful monitor of immune status. T cell function can be monitored by the ability of the T cells to produce IFN-γ. This is currently being used to identify tuberculosis (TB) reactivity and is discussed later.
Third, recombinant cytokines are key therapeutic agents. An example of this is seen with the IFN molecules. The FDA has approved the use of IFN-α for hepatitis C infections, IFN-β for multiple sclerosis, and IFN-γ for chronic granulomas disease (CGD).
Fourth, cytokines can be targets of therapy. Recently, cytokine receptor antagonists and anticytokine monoclonal antibodies that downregulate pathogenic responses to exaggerated cytokine production have been used as effective treatments. Examples of this approach are the inhibitors of TNF-α used to manage rheumatoid arthritis (RA) and inhibitors of IL-2 and IL-15 used in transplantation and cancer.

HYPERSENSITIVITY
Hypersensitivity is a condition in which an exaggerated or augmented immune response occurs that is harmful to the host. Hypersensitivity requires a presensitized state. For example, in a given individual, such reactions typically occur after the second encounter with that specific antigen (allergen).
In 1963, Coombs and Gell classified hypersensitivity into four types: Types I, II, III (antibody mediated), and IV (T cell mediated).

Type I: Immediate Hypersensitivity (Allergy)
Type I hypersensitivity manifests itself in tissue reactions occurring within seconds after the antigen combines with specific IgE antibody. Its symptoms may manifest as a systemic anaphylaxis (e.g., after intravenous administration of heterologous proteins) or as a local reaction (e.g., an atopic allergy involving rhinitis such as occurs with hay fever).
The general mechanism of immediate hypersensitivity involves a series of steps. An antigen induces the formation of IgE antibody, which binds firmly by its Fc portion to high-affinity IgE receptors on mast cells, basophils, and possibly eosinophils. Some time later, an individual experiences a second exposure to same antigen. This second exposure results in the cross-linking of the cell bound IgE molecules and the release of pharmacologically active mediators. Cyclic nucleotides and calcium are essential in the release of mediators.
Pharmacologic mediators of type I hypersensitivity are listed as follows:

1. Histamine—Histamine exists in a preformed state in platelets and in granules of mast cells, basophils, and eosinophils. The release of histamine causes vasodilation, increased capillary permeability, and smooth muscle contraction (e.g., bronchospasm). Antihistamine drugs can

block histamine receptor sites and are relatively effective in allergic rhinitis. Histamine is one of the primary mediators of a type I reaction.

2. Prostaglandins and leukotrienes—Prostaglandins and leukotrienes are newly formed mediators derived from arachidonic acid via the cyclooxygenase pathway. Prostaglandins induce edema and bronchoconstriction. Leukotriene B4 is a chemoattractant that activates and recruits leukocytes to the site of injury. Leukotrienes C4 and D4 cause vasodilation and vascular permeability. These mediators, along with TNF-α and IL-4, are referred to as secondary mediators of a type I reaction.

A. Atopy

Atopic hypersensitivity disorders exhibit a strong familial predisposition and are associated with elevated IgE levels. Predisposition to atopy is clearly genetic, but the symptoms are induced by exposure to specific allergens. These antigens are typically environmental (e.g., respiratory allergy to pollens, ragweed, or house dust) or foods (e.g., intestinal allergy to shellfish). Common clinical manifestations include hay fever, asthma, eczema, and urticaria. Many patients experience immediate-type reactions to skin tests (injection, patch, scratch) involving the offending antigen.

B. Treatment and Prevention of Anaphylactic Reactions

Treatment aims to reverse the action of mediators by maintaining the airway, providing artificial ventilation if necessary, and supporting cardiac function. Epinephrine, antihistamines, and corticosteroids are often given. However, the best prevention relies on the identification of the antigen (detected by skin test or IgE antibody serology) and subsequent avoidance.

Type II: Hypersensitivity

Type II hypersensitivity involves the binding of IgG antibodies to cell surface antigens or extracellular matrix molecules. Antibody directed at cell surface antigens can activate complement to damage the cells. The result may be complement-mediated lysis, which occurs in hemolytic anemia, ABO transfusion reactions, and Rh hemolytic disease.

Drugs such as penicillin can attach to surface proteins on red blood cells and initiate antibody formation. Such autoimmune antibodies may then combine with the cell surface, and cause hemolysis. In Goodpasture syndrome, antibody is generated to the basement membranes of the kidney and lung. This results in complement activation, leukocyte chemotaxis, and severe membrane damage. In some cases, antibodies to cell surface receptors alter cell function without cell injury (e.g., in Graves disease, an autoantibody binds to the thyroid-stimulating hormone receptor, which generates stimulation of the thyroid, and hyperthyroidism).

Type III: Immune Complex Hypersensitivity

When antibody combines with its specific antigen, immune complexes are formed. Normally, these immune complexes are promptly removed, but occasionally, they persist and are deposited in tissues. In persistent microbial or viral infections, immune complexes may be deposited in organs (e.g., the kidneys), resulting in tissue and organ dysfunction. In autoimmune disorders, "self" antigens may elicit antibodies that bind to organ antigens or are deposited in organs and tissues as complexes, especially in the joints (arthritis), kidneys (nephritis), and blood vessels (vasculitis). Finally, environmental antigens such as fungal spores and certain drugs can cause immune complex formation with similar tissue and organ damage.

Wherever immune complexes are deposited, they can activate complement. Once complement is activated, macrophages and neutrophils migrate to the site and inflammation and tissue injury ensue. There are two major forms of immune complex-mediated hypersensitivity. One type of

immune complex-mediated hypersensitivity is produced locally and is called Arthus reaction. This reaction occurs when a low dose of antigen is injected into the skin. This induces the production of IgG antibodies and complement activation. In addition, mast cells and neutrophils are stimulated to release their mediators that enhance vascular permeability. This reaction usually occurs within 12 hours.

Another example of type III hypersensitivity involves a systemic immune complex disease such as acute poststreptococcal glomerulonephritis.

Acute poststreptococcal glomerulonephritis is a well-known immune complex disease. Its onset takes place several weeks after a group A β-hemolytic streptococcal infection, particularly of the skin, and often occurs with infection due to nephritogenic types of streptococci. The complement level is typically low, suggesting an antigen–antibody reaction with consumption of complement. Lumpy deposits of immunoglobulin and complement component, C3, are observed along the glomerular basement membrane. These membranes can be stained by immunofluorescence and visualized under UV microscopy. This type of pattern reveals antigen–antibody complexes. It is likely that streptococcal antigen–antibody complexes are filtered out by glomeruli, fix complement, and attract neutrophils. This series of events results in an inflammatory process that damages the kidney.

Type IV: Cell-Mediated (Delayed) Hypersensitivity

Cell-mediated hypersensitivity is a T cell–mediated response. The interaction of an antigen with specifically sensitized T cells results in T cell proliferation, release of potent inflammatory cytokines (IFN-γ and IL-2), and activation of macrophages. This inflammatory response most often begins 2 or 3 days after contact with the antigen and typically lasts for several days.

A. Contact Hypersensitivity

Contact hypersensitivity occurs after sensitization with simple chemicals (eg, nickel, formaldehyde), plant materials (poison ivy, poison oak), topically applied medications (eg, sulfonamides, neomycin), some cosmetics, soaps, and other substances. In all cases, small molecules enter the skin and then, acting as haptens, attach to body proteins to serve as complete antigens. Cell-mediated hypersensitivity is induced, particularly in the skin. When the skin again comes in contact with the offending agent, the sensitized person develops erythema, itching, vesication, eczema, or necrosis of skin within 12–48 hours. Avoidance of the inciting material will prevent recurrences. A skin test may identify the antigen in question. Langerhans cells in the epidermis, which interact with CD4 Th1 cells, appear to play a role in driving this response.

B. Tuberculin-Type Hypersensitivity

Delayed hypersensitivity to antigens of microorganisms occurs in many infectious diseases and it has been used as an aid in diagnosis. The tuberculin reaction is a good example of a delayed-type hypersensitivity (DTH) response. When a small amount of tuberculin is injected into the epidermis of a patient previously exposed to Mycobacterium tuberculosis, there is little immediate reaction. Gradually, however, induration and redness develop and reach a peak in 24–72 hours. Mononuclear cells, especially CD4 Th1 cells, accumulate in the subcutaneous tissue. A positive skin test indicates that the person has been infected with the microorganism but does not imply the presence of current disease. However, a recent change of skin test response from negative to positive suggests recent infection and possible current activity.