Function and Evaluation of the Immune System

Philip D. Hall and Nicole Weimert Pilch

1 Cells of the immune system are derived from the pluripotent stem cell. Hematopoiesis is closely regulated to assure adequate amounts of each of the different types of blood cells. The development of the different kinds of cells or cell lineages depends on cell-to-cell interactions and hematopoietic growth factors.

2 After activation, dendritic cells (DCs) express higher concentrations of major histocompatibility complex (MHC) class II molecules, B7-1, B7-2, CD40, ICAM-1, and LFA-3 molecules than other antigen-presenting cells (APCs). They also produce more IL-12. These differences may explain why in vitro DCs are the most efficient APC.

3 A T lymphocyte expresses hundreds of T-cell receptors (TCRs). All the TCRs expressed on the surface of an individual T lymphocyte have the same antigen specificity.

4 A B lymphocyte can simultaneously express membrane immunoglobulin as IgM (monomeric) or IgD with the same variable region (i.e., antigen-binding site). The B lymphocyte then can secrete different isotypes (e.g., IgM [pentamer], IgA, immunoglobulin G [IgG], and IgE) with the same variable region as the membrane immunoglobulin.

5 A serum protein electrophoresis (SPEP) determines the total concentration of circulating immunoglobulins (i.e., IgG, IgA, IgM, IgD, and IgE). If one wishes to determine the concentration of the individual isotypes, one needs to order isotype quantification. Most clinical laboratories quantitate only IgG, IgM, and IgA because they are the most prevalent isotypes in the bloodstream. In patients with allergic disorders, quantification of IgE may be useful. Depending on the clinical laboratory, results may come back in IU/mL, kIU/L, or mg/L for IgE.

6 An understanding of the mechanism by which immunomodulators act along with an understanding of the immune system allows a clinician to anticipate potential adverse effects. The benefit of manipulating the immune responses must be balanced with the potential consequences and long-term sequela of such manipulation.

The immune system is a complex network of barriers, organs, cellular elements, and molecules that interact to defend the body against invading pathogens. The immune system is actually composed of two distinct systems of immunity: innate immunity and adaptive immunity. In brief, innate immunity includes a series of nonspecific barriers (physical and chemical), along with cellular and molecular elements strategically predeployed and prepositioned to prevent and/or quickly neutralize infection. Working in concert with innate immunity is adaptive immunity. In contrast to innate immunity, adaptive immunity constantly evolves and adapts against invading pathogens. Its hallmarks include diversity, memory, mobility, self versus non-self discrimination, redundancy, replication, and specificity.1 Diversity indicates the capability of the immune system to respond to many different pathogens or strains of pathogens. Immunological memory ensures a quicker and more vigorous response to a subsequent encounter with the same pathogen. Mobility of components of the immune system enables local reactions to provide systemic protection. Discrimination of self versus non-self helps prevent friendly fire damage of the host by the immune system. Redundancy refers to the immune system’s ability to produce components with similar biological effects from multiple cells lines, such as inflammatory cytokines. Replication of the cellular components of the immune system amplifies the immune response. Specificity describes the ability of the immune system to distinguish between dissimilar antigens.

MAJOR TISSUES AND ORGANS OF THE IMMUNE SYSTEM

While numerous cells of the immune system have the ability to migrate to most body tissues, some tissues and organs serve as key members of the immune system. These include primary and secondary lymphoid tissues and organs. Primary lymphoid tissues and organs provide an environment appropriate for the development and maturation of select cells of the immune system. It is here that these select cells of the immune system mature and become tolerant of self and competent to respond to foreign antigens. Secondary lymphoid organs provide an environment where various cells of the immune system interact with and respond to trapped foreign antigens.2

Bone Marrow

1 Bone marrow is the predominant primary lymphoid tissue of the body because it is the source of all cellular elements of the blood (erythrocytes, leukocytes, and thrombocytes [i.e., platelets]). The few exceptions to this rule are mostly confined to fetal development when some blood cells are transiently produced in the yolk sac, liver, spleen, thymus, and lymph nodes.3 Regardless of where they are formed, all blood cells arise from common self-renewing pluripotent stem cells via the process of hematopoiesis (eFig. 21-1). During hematopoiesis, pluripotent stem cells differentiate along particular myeloid and lymphoid lineages to produce the leukocytes of the immune system, erythrocytes, and thrombocytes.4,5 Hematopoiesis is controlled by soluble mediators called hematopoietic growth factors/cytokines or colony-stimulating factors (CSFs) that are multifunctional and drive responses such as growth, survival, proliferation, differentiation, maturation, and functional activation.6
immunity, including cytotoxic actions and immunoregulation. Through an intricate multistep process called thymic education, T cells that respond appropriately and are beneficial to the immune system mature and leave the thymus. T cells that fail the thymic education test (99%) are eliminated via apoptosis.1

Spleen

The spleen is a slender elongated secondary lymphoid organ located in the upper left quadrant of the abdomen that receives blood from the splenic artery. Although it is not a vital organ, it functions as an immunological filter of the blood and destroys defective and old erythrocytes. It contains compartments designated as red pulp and white pulp and provides an environment for the interaction of its filtered debris from blood with APCs and lymphocytes responsible for cell-mediated responses (T cells) and lymphocytes responsible for antibody production (B cells). Red pulp serves as a site of red blood cell degradation, and the white pulp provides an environment for immune responses.

Thymus

The thymus is a bi-lobed primary lymphoid organ located in the superior mediastinum between the aorta and the sternum. Its primary function is to produce mature T cells (thymus-dependent lymphocytes), which are the leukocytes responsible for cell-mediated immunity, including cytotoxic actions and immunoregulation. Through an intricate multistep process called thymic education, T cells that respond appropriately and are beneficial to the immune system mature and leave the thymus. T cells that fail the thymic education test (99%) are eliminated via apoptosis.1

### eFIGURE 21-1
Basic model of hematopoiesis, outlining the various pathways blood cells take from their origin as bone marrow stem cells through stages in which they are progressively selected to become monopotent mature cells with specific functions. Selected hematopoietic growth factors include: EPO, erythropoietin; FLT-3L, fms-like tyrosine kinase ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; NK, natural killer; SCF, stem cell factor; TPO, thrombopoietin.
for the interaction of B cells and T cells. The spleen sequesters many cellular elements (leukocytes, erythrocytes, and platelets) and can become dangerously congested in a condition termed splenomegaly.

**Lymph Nodes**

Lymph nodes are normally small BB-sized lymphoid organs widely distributed between the groin and the neck. While the spleen filters blood, lymph nodes act as immunological filters for interstitial lymphatic fluid from the body’s tissues. Lymph nodes provide an environment for the interaction of filtered debris with APCs and other immune cells (T cells and B cells). Lymph nodes may sequester activated immune cells (or tumor cells) and become inflamed and engorged causing lymphadenopathy.

**Mucosa-Associated Lymphoid Tissue**

Mucosa-associated lymphoid tissue (MALT) is the most extensive component of human lymphoid tissue and is distributed along mucosal linings of the body. MALT may consist of well-defined networks of lymphoid cells that are found in intestinal villi. The primary function of these tissues is to filter, trap, and remove pathogens that breach mucosal surfaces. In addition to neutralizing pathogens, MALT generates plasma cells (activated B cells) that secrete IgA antibodies.

As mentioned earlier, the immune system includes two functional divisions: (a) the innate or nonspecific which encodes evolutionary genes aimed at providing rapid responses against non-mammalian targets and (b) the adaptive or specific which utilizes cells which can genetically rearrange their DNA to create specific structures which bind individual antigens or proteins (eTable 21-2).

Despite this simple separation, these divisions extensively interact. Awareness of each component of the immune system and the consequences of disrupting homeostasis must be understood in order to appropriately dose, administer, and monitor the effect of medications given to manipulate immune responses.

### METHODS TO DISTINGUISH SELF FROM NONSELF

The immune system is designed to attack and destroy a broad spectrum of foreign antigens/pathogens. The immune system must, however, be able to distinguish self from nonself, termed self-tolerance, in order to avoid unleashing its components onto self-tissues. The body employs many tactics to avoid attacking itself, and when self-tolerance fails this may lead to the development of an autoimmune disease.

**Innate Immune System**

**Physical Defense**

Physical and chemical defenses are the most rudimentary form of innate immunity and the first line of defense against invading pathogens. The skin, the largest organ of the body, has the primary role of providing a physical defense. Alterations in the skin, such as burns or abrasions, allow an easy portal of entry for pathogens. The GI tract also provides physical defense. The low pH of the stomach (pH 1 to 2) is inhospitable to most organisms. The rapid turnover of intestinal cells also limits systemic infection as cells including infected cells are sloughed frequently. Drugs, such as cell-cycle, phase-specific antineoplastics that disrupt the sloughing process, leave the patient at an increased risk for infections. Likewise, the respiratory tract has its forms of physical defense. The mucus coating the epithelial cells serves in part to prevent microorganisms from adhering to cell surfaces, and the cilia lining the epithelium of the lungs help to repel inhaled organisms. The combination of cilia, mucus, and reactive coughing provides a natural barrier to invasion via the respiratory tract. Other examples of mechanical or nonspecific defenses include normal urine flow, lysozymes in tears and saliva, and the normal flora in the throat, the lower GI tract, and the genitourinary tract. Disruption of the normal physical defense system through mechanical ventilation, for example, places the host at substantial risk for penetration by a pathogenic organism.

**Innate Immune Response**

If an infectious pathogen invades and is able to infiltrate through a host’s physical defense system, innate immunity is employed to...
Organ-Specific Function Tests and Drug-Induced Diseases

Section

mannose receptor, macrophage scavenger receptor, and members of the toll-like receptor family. Pattern recognition receptors include toll-like receptors, scavenger receptors, and mannose receptors.

halt progression of the infection. Innate immunity is present from birth and utilizes a preexisting but limited repertoire of receptors to recognize and destroy pathogens. Innate immune cells include subgroups of leukocytes; specifically, monocytes/macrophages, neutrophils, basophils, mast cells, and eosinophils. When stimulated by a foreign pathogen, mast cells and basophils secrete inflammatory mediators. Monocytes/macrophages, neutrophils, mast cells, and eosinophils act as phagocytes, which allow them to recognize, internalize, and destroy invading pathogens. This process may occur in two ways: opsonin-dependent or opsonin-independent phagocytosis. For opsonin-dependent phagocytosis, antibody (e.g., IgG), complement (e.g., C3b), or lectin (e.g., C-reactive protein) coat, or opsonize, the infectious pathogens. Once the pathogen is opsonized, the antibody, complement, or lectin binds to the receptors on the phagocyte (eFig. 21-2) and activates the phagocytic process. For opsonin-independent phagocytosis, innate leukocytes utilize pattern recognition receptors. Pattern recognition receptors recognize highly conserved structures present on a large number of microorganisms. These highly conserved structures are essential for the microorganism’s survival or pathogenicity. The pattern recognition receptors include the macrophage mannose receptor, macrophage scavenger receptor, and members of the toll-like receptor family. Pattern recognition receptors on the phagocytes directly recognize ligands (eTable 21-3) on the surfaces of infectious pathogens leading to immediate phagocytosis of the pathogen (eFig. 21-2). Toll-like receptors are a family of pattern recognition receptors on the cell-surface of innate leukocytes. To date, 11 toll-like receptors have been identified in humans. They recognize a broad spectrum of antigens ranging from lipopolysaccharide and flagellin on bacteria to zymosan on yeast to double-stranded RNA from RNA viruses (eTable 21-3). Binding of the ligand to the toll-like receptors allows the phagocyte to recognize and engulf the pathogen. This binding of toll-like receptors to its ligand also results in the secretion of chemokines, inflammatory cytokines, and antimicrobial peptides as well as the increased expression of costimulatory proteins (e.g., B7) and the MHC proteins by the phagocyte. This leads to the recruitment and activation of antigen-specific lymphocytes. Other pattern recognition receptors that mediate phagocytosis include macrophage receptor with collagenous structure (MARCO), DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin), and dectin 1.

Cells of the Innate Immune System

Neutrophils, eosinophils, and basophils are considered granulocytes because of the presence of numerous cytoplasm granules in these cells that contain inflammatory mediators or digestive enzymes. Their names are derived from their staining characteristics; neutrophils are named because they stain a neutral pink. Neutrophils comprise the majority of leukocytes in the bloodstream. They are polymorphonuclear cells, often denoted as PMNs for this reason, which serve as the primary human defense against invasive bacteria. Neutrophils migrate from the bloodstream into infected or inflamed tissue in response to chemotactic factors, such as IL-8 and C3a and C5a, breakdown products of complement. In this migration, a process termed chemotaxis, neutrophils reach the site of inflammation and then recognize, adhere to, and phagocytose pathogens. Via the complement and antibody receptors located on its surface, neutrophils can recognize and engulf pathogens opsonized with complement or IgG (antibody). During phagocytosis, the engulfed pathogen is internalized within the phagocyte into a cytoplasmic lysosome. The neutrophil then releases its granular contents into lysosome and generates the release of oxidative metabolites that destroy the engulfed pathogens. Neutrophils can also recognize pathogens via toll-like receptors.

Eosinophils are also granulocytic cells involved in innate immunity. They exhibit motility and migrate from the blood into the tissues. They play a less significant role in combating bacterial infections, but eosinophils play a major role against nonphagocyttable multicellular pathogens, such as parasites. After activation via high-affinity receptor for IgE (i.e., FcεR), eosinophils exocytose their granules causing the release of basic proteins or reactive oxygen species into the microenvironment, causing lysis of the parasite. In addition to FcεR receptors, eosinophils express lower levels of complement receptor 3 and FcγR for IgG than neutrophils. The high affinity of eosinophils for IgE contributes to their role in the pathogenesis of allergic disorders (i.e., allergic asthma).

Macrophages and monocytes are mononuclear cells capable of phagocytosis. Tissue macrophages arise from the migration of monocytes from the bloodstream into the tissues. Macrophages differ from monocytes by possessing an increased number of Fc and complement receptors. Macrophages are found within specific tissues such as the liver, spleen, GI tract, lymph nodes, brain, and others. These specific types of macrophages are often called histiocytes, or referred to by a specialized name depending on the site where they are found (for example, Kupffer cells in the liver, osteoclasts in the bone, and microglial cells in the CNS). The term reticuloendothelial system (RES) was commonly used to refer to macrophages found in reticular connective tissue, but the preferred nomenclature is now the mononuclear phagocyte system.

eTable 21-3 Ligands for Pattern Recognition Receptors

<table>
<thead>
<tr>
<th>Pathogen Ligand</th>
<th>Type of Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoteichoic acid</td>
<td>Gram-positive organisms</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>Gram-negative organisms</td>
</tr>
<tr>
<td>Mannose</td>
<td>Fungi, gram-positive, gram-negative</td>
</tr>
<tr>
<td>Double-stranded RNA</td>
<td>RNA viruses</td>
</tr>
<tr>
<td>Triacyl lipopeptides</td>
<td>Gram-positive, gram-negative</td>
</tr>
<tr>
<td>Peptidoglycans</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>Bacterial flagella</td>
<td>Various</td>
</tr>
</tbody>
</table>

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Despite the first description in 1868 of Langerhans cells, a type of DC found in the skin, our current understanding of the biologic function of DCs did not develop until the past decade. Before pathogen recognition, most DCs are in an immature/resting state with limited ability to activate T lymphocytes, but they express numerous receptors (e.g., Fc receptors of IgG and IgE, macrophage mannose receptor, and toll-like receptors) enabling rapid antigen recognition. Following antigen recognition and particle engulfment, DCs become activated. This leads to a dramatic increase in their expression of the MHC class II, B7, CD40, and adhesion molecules. DCs then begin to migrate through the tissues toward lymphoid organs (e.g., spleen, lymph nodes) to present antigen to T lymphocytes, causing activation of the adaptive immune system.14

In addition to phagocytosing pathogens, macrophages and DCs act as APCs to stimulate the adaptive (specific) system. Macrophages and DCs internalize the organism, digest it into small peptide fragments, and then combine these antigenic fragments with MHC proteins. Once the APC has formed the antigen/MHC complex, the APC places the complex on its surface. This surface complex can then be recognized by the TCR on the surface of a T lymphocyte. The recognition of the antigen/MHC complex by the TCR is the first step in the activation of the T lymphocyte. The binding of CD2-CD58 and LFA-1 (CD11a/CD18) allows adherence between the T lymphocyte and DC. Upon activation, the TH1 CD4+ T lymphocyte secretes IL-2 and interferon (IFN)-γ and increases the production and expression of the IL-2 receptor. (ICAM, intercellular adhesion molecule).

eFIGURE 21-3 Induction of T-helper type 1 (TH1) response. 1. The APC, in this case a DC, engulfs the pathogen by any of numerous cell surface receptors (eFig. 21-2). After phagocytosis of the bacteria by the DC (A), the pathogen is digested into small peptides and become associated with major histocompatibility (MHC) class II within the endosome (B). Finally, the MHC class II plus peptide is expressed on the surface of the DC (C). The activated DC also secretes interleukin (IL)-12. 2. Naive CD4+ T-lymphocyte activation requires the T-cell receptor (TCR) to recognize the antigenic peptide in association with MHC class II as well as the B7-1 (CD80) binding to CD28. The binding of CD2-CD58 and LFA-1 (CD11a/CD18) allows adherence between the T lymphocyte and DC. Upon activation, the TH1 CD4+ T lymphocyte secretes IL-2 and interferon (IFN)-γ and increases the production and expression of the IL-2 receptor. (ICAM, intercellular adhesion molecule).

SOLUBLE MEDIATORS OF THE INNATE IMMUNE SYSTEM

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in immune defense. The four major functions of the complement system include (a) to lyse certain microorganisms and cells, (b) to stimulate the chemotaxis of phagocytic cells, (c) to coat or opsonize foreign pathogens, which allows phagocytosis of the pathogen by leukocytes expressing complement receptors, and (d) to clear immune complexes. Complement factors (C3a, C5a) act as chemotactic factors for phagocytic cells. Two different pathways stimulate the complement cascade. In the classic pathway, antibody binds to its target antigen and activates the first component of complement (C1), thereby initiating the complement cascade. The alternative complement pathway relies on the inability of microorganisms to clear spontaneously produced C3b, the active form of third complement protein, from their surface. Patients with hereditary deficiencies of complement have recurrent bacterial infections or immune complex disease because C3b plays a central role in opsonizing bacteria and clearing immune complexes. Both mannan-binding lectin and CRP are acute-phase reactants produced by the liver during the early stages of an infection. They bind to infectious pathogens that prompt the activation of the lectin or minor pathway of the complement system. Mannan-binding lectin binds to mannose-rich glycoconjugates on microorganisms while CRP binds to phosphocholine on bacterial surfaces.

Chemokines play an essential role in linking the innate and adaptive immune response by orchestrating leukocyte trafficking. The chemokine system consists of a group of small polypeptides and their receptors. Chemokines possess four conserved cysteines. Based on the positions of the cysteines, almost all chemokines fall into one of two categories: (a) CC group in which the conserved cysteines are contiguous or (b) CXC subgroup in which the cysteines are separated by some other amino acid (“X”). As with all ligand–receptor interactions, a cell can only respond to a chemokine if the cell possesses a receptor that recognizes the chemokine. Chemokine receptors are unique in that they traverse the membrane seven times. CC receptors (CCR) and CXC receptors (CXCR) bind CC ligands (CCL) and CXC ligands (CXCL), respectively (eTable 21-4).

Binding of infectious pathogens to pattern recognition receptors stimulates the release of chemokines such as macrophage inflammatory protein (MIP)-1α, MIP-1β, MIP-3α, and IP-10 from macrophages and DCs embedded in the tissues. These chemokines attract more immature DCs to the site of inflammation/infection. Immature DCs constitutively express CCR1, CCR5, and CCR6. The interaction between pattern recognition receptors on the DC to the infectious pathogen causes the activation and maturation of the DC. After activation, DCs downregulate the expression of CCR1, CCR5, and CCR6 and upregulate the expression of CCR7. This switch in chemokine-receptor expression results in the antigen-loaded DC leaving the tissue and migrating toward the lymph nodes.

Naturally occurring antimicrobial peptides include α-defensins, β-defensins, and cathelicidins. These peptides exhibit antibacterial, antifungal, and antiviral activity. Human antimicrobial peptides range in size from 29 to 37 amino acid residues in length. Neutrophils are rich source of both α- and β-defensins as well as cathelicidins. Other sources of the human antimicrobial peptides include keratinocytes, paneth cells of the intestinal and genital tracts, and epithelial cells of the pancreas and the kidney. These peptides can be induced at sites of inflammation or can be constitutively produced. The clinical interest in human antimicrobial peptides centers on their broad-spectrum activity and their rapid onset of killing. They are believed to work by disrupting microbial membranes. An active area of research is how these peptides discriminate between microbial and host membranes.

Adaptive Immune System
Adaptive Immune Response: Antigen Recognition

The body will generally employ both the innate and adaptive immune responses to rapidly kill foreign pathogens. The greatest difference between the innate and adaptive immune responses is in specificity and memory, characterized by antigen-specific receptors located on the surface of B and T lymphocytes. The adaptive immune response also secretes cytokines to further amplify the innate immune response. The adaptive immune response can evolve with each subsequent infection whereas the innate response stays the same with each infection. During B- and T-lymphocyte development, an individual B or T lymphocyte rearranges its immunoglobulin or TCR genes, respectively, to produce a unique immunoglobulin or TCR, respectively. This DNA rearrangement generates enough B or T lymphocytes to recognize an estimated 10^13 antigens.

The adaptive immune response can be divided into two major arms: humoral and cellular mediated. The humoral response is so denoted because it was discovered that the factors that provided the immune protection could be found in the “humor” or serum. B lymphocytes comprise the humoral arm. Activated B lymphocytes can differentiate into plasma cells that secrete immunoglobulin or memory B cells specific for each pathogen. T lymphocytes constitute the cell-mediated arm of the adaptive system. The immune protection provided by T lymphocytes cannot be transferred by serum alone. Rather, it is essential to actually have T lymphocytes present, thus the term cell-mediated immunity. T lymphocytes are specially tailored to defend against infections that are intracellular, such as viral infections, whereas B lymphocytes secrete antibodies that can neutralize pathogens prior to their entry into host cells.

Adaptive Immune Response: Cells Which Mediate Antigen Recognition

The role of the T lymphocyte is to search and destroy pathogens that infect and replicate intracellularly. When these pathogens enter a cell they are no longer vulnerable to innate host defenses; therefore, it is critical that the T lymphocytes be able to distinguish which cells are infected and which cells are not. T lymphocytes do not recognize intact antigens, such as a bacterial cell wall. T lymphocytes only recognize processed antigens in association with MHC.

The major histocompatibility complex, a cluster of genes found on chromosome 6 in humans, is also known as the human leukocyte antigen (HLA) complex. The MHC is used by the immune system to distinguish self from nonself and provides a so-called immunologic “fingerprint.” The genes from this complex encode for molecules that play a pivotal role in immune recognition and response. The MHC complex is divided into three different classes: I, II, and III. The molecules encoded by class I HLA genes include HLA-A,
HLA-B, and HLA-C antigens. These molecules can be found on all nucleated cells within the body, as well as on platelets. Class I antigens are not found on mature red blood cells. Molecules encoded by class II HLA genes include HLA-DP, HLA-DQ, and HLA-DR. The expression of these molecules is more restricted and can be found primarily on cells of the immune system, namely APCs such as macrophages, DCs, and B lymphocytes. The class III HLA antigens encode for soluble factors, complement, and tumor necrosis factors. In order for a CD4+ T lymphocyte to become activated, CD4+ T lymphocyte must recognize the antigenic peptide in association with MHC class II (eFigs. 21-3 and 21-4). CD8+ T lymphocytes recognize antigenic peptide in association with class I molecules. Class I molecules generally contain endogenous peptides from within the cell, such as those derived from viruses, while class II molecules contain exogenous peptides from antigen that has been phagocytosed and digested, such as bacterial peptides (eFig. 21-3). For it to destroy a virally infected cell, a CD8+ cytotoxic T lymphocyte requires two steps. First, its TCR must recognize the antigenic fragment, such as a viral protein, in association with MHC class I. The second step involves the costimulatory step of B7-CD28 binding. This process is further defined below. Because any cell can become infected, it is advantageous that the CD8+ T lymphocyte to propagate the immune response. The TCR is comprised of single chains known as the CD3 complex. APCs (e.g., macrophages, DCs) engulf the pathogen, digest it, and express its peptide fragments on their cell surface in association with their MHC. T lymphocytes use a specific antigen receptor, TCR, to propagate the immune response. The TCR is comprised of two chains with each chain having a variable and a constant region. The variation of the amino acid sequence within the variable domain of TCR gives the cell its unique antigen specificity. Linked to the TCR is a complex of single chains known as the CD3 complex. Macrophages and other cells of the immune system (eFig. 21-5B). This model of CD8+ cytotoxic T lymphocyte activation requires the close proximity of two-antigen specific T lymphocytes. In addition, some CD8+ cytotoxic T lymphocytes activate/prime APCs through CD40. This interaction primes the APC to fully activate CD8+ cytotoxic T lymphocytes (eFig. 21-5B). It is important to remember that the classification of CD4+ T lymphocytes as T-helper lymphocytes and CD8+ T lymphocytes are cells that have not been previously exposed to an antigen specific for their TCR. These cells require two signals for activation. The first signal for activation involves the T lymphocyte recognizing both the processed antigen and the MHC molecule complex. The second signal involves the interaction of the B7-1 (CD80) or B7-2 (CD86) molecule on the APC with the CD28 molecule on the surface of the T lymphocyte (eFigs. 21-3 and 21-4). Without the second signal, the naïve T lymphocyte becomes anergic or inactive. Memory T lymphocytes are less dependent on the second signal than are naïve T lymphocytes. CD28 is expressed on both resting and activated T lymphocytes while CTLA-4, a second ligand for B7 on T lymphocytes, is expressed only on activated T lymphocytes. CTLA-4 binding B7 transduces a negative signal so it plays a role in downregulating a T-lymphocyte response. After the two activation signals, a message is sent through the TCR to the CD3 complex into the cell. Then a calcium influx occurs with subsequent activation of the T lymphocyte. Activated CD4+ T lymphocytes begin to express the high-affinity IL-2 receptor and to release multiple soluble factors (e.g., IL-2) to stimulate T lymphocytes and other cells of the immune system (eFig. 21-3). Autoimmune stimulation by IL-2 leads to the proliferation of the activated T lymphocyte.

Cell surface markers delineate the functional activity of T-lymphocyte populations. All T lymphocytes express the CD3 protein. Typically, T lymphocytes are further divided into helper cells (CD4+), suppressor cells (CD8+), and cytotoxic cells (CD8-). Each of the subclasses appears to play a distinct role in the cell-mediated immune response. Naïve T lymphocytes express CD45RA, a high-molecular-weight isofrom of CD45, while memory T lymphocytes express CD45RO, a lower-molecular-weight isofrom of CD45. The primary role of CD4+ cells is to stimulate other cells in the immune response. Functionally, CD4+ cells can be divided into T-helper type 1 (TH1), T-helper type 2 (TH2), TH1p, TH2p, and T-regulatory (Tregs). This functional system was first described in mice. TH1 cells secrete IL-2 and γ interferon and stimulate CD8+ cytotoxic cells, whereas TH2 cells secrete IL-4, IL-5, and IL-10 and stimulate B-lymphocyte production of antibody toward extracellular pathogens. Multiple factors determine whether a naïve CD4+ T lymphocyte develops into a TH1 or a TH2 cell. The cytokine microenvironment plays an important role in this development. IL-12 secreted by the APCs promotes TH1, whereas IL-4 promotes TH2 development. Other factors that promote TH1 development include B7-1 (CD80), high-affinity of the TCR for the antigen, γ interferon, and α interferon. Factors that promote TH2 development include B7-2 (CD86), low-affinity of the TCR for the antigen, IL-10, and IL-1. TH17 are also promote B-lymphocyte activation and play a crucial role in generation of memory B-lymphocytes which leads to long-lived antibody responses. The TH17 subset were discovered because of selective production of IL-17 and play an important role in immunity in mucosal tissues. They may play a significant role in the pathogenesis of multiple inflammatory and autoimmune disorders.

CD8+ T lymphocytes recognize antigen in association with MHC class I. CD8+ cytotoxic cells are instrumental in killing cells recognized as foreign, such as those that have become infected by a virus. CD8+ cytotoxic T lymphocytes also play an important beneficial role in the eradication of tumor cells, but moreover are responsible for rejection of transplanted organs. Classically, a second type of CD8+ T lymphocytes was a suppressor cell. It is clear that some T lymphocytes help suppress the immune responses, but whether this subset is CD8+ is debatable. Emerging evidence is leading away from CD8+ T lymphocytes toward CD4+, CD25+ T lymphocytes in maintaining self-tolerance. The preferred term for these suppressive T lymphocytes is regulatory T lymphocytes.

To fully activate the CD8+ cytotoxic T lymphocyte requires CD4+ T lymphocyte activation, namely the TH1 subset, and its subsequent secretion of IL-2 (eFig. 21-5A). This model of CD8+ cytotoxic T lymphocyte activation requires the close proximity of two-antigen specific T lymphocytes. In addition, some CD8+ cytotoxic T-lymphocyte responses can occur in the absence of CD4+ T lymphocytes. New data suggest that CD4+ T-lymphocytes can activate/prime APCs through CD40. This interaction primes the APC (e.g., DC) to fully activate CD8+ cytotoxic T lymphocytes (eFig. 21-5B). It is important to remember that the classification of CD4+ lymphocytes as T-helper lymphocytes and CD8+ lymphocytes as T-cytotoxic lymphocytes is not an absolute. Some CD8+ T lymphocytes secrete cytokines similar to a T-helper lymphocyte, and some CD4+ T lymphocytes can act as cytotoxic cells.

Unlike neutrophils and macrophages, cytotoxic T lymphocytes are unable to ingest their targets. They destroy target cells by two different mechanisms: the perforin system and the Fas ligand pathway. After recognition by the cytotoxic T lymphocyte, cytoplasmic granules containing perforins and granzymes are rapidly oriented toward the target cell, and the contents of the granules are released into the intracellular space. Like the membrane attack complex formed after complement activation, perforins form a pore in the target cell membrane. Besides a direct cytotoxic effect on the target cell, the pores produced by perforins allow the granzymes to penetrate into the target cell to induce apoptosis. The second mechanism of cytotoxicity involves the binding of Fas ligand (FasL) on the cytotoxic T lymphocyte to the Fas receptor on the target cell. The FasL is predominately expressed on CD8+ cytotoxic T lymphocytes and natural killer (NK) cells, and its expression increases after activation. After destroying that target cell by either mechanism, the cytotoxic T lymphocyte detaches from the target cell and attacks other targets.
A B lymphocyte recognizes antigen via its antibody or immunoglobulin located on its cell surface (eFig. 21-4). The antibody on the surface can recognize an intact pathogen, such as bacteria, and present antigen to T lymphocytes (i.e., acting as APC). However, the major function of B lymphocytes is to produce antibody to bind to the invading pathogen, a process that first entails activation of the B lymphocyte. The activation of B lymphocytes also requires two steps: (a) recognition of antigen by the surface immunoglobulin and (b) the presence of B-lymphocyte growth factors (IL-4, -5, and -6) secreted by activated CD4+ T lymphocytes. Once activated, the B lymphocyte becomes a plasma cell, a differentiated cell capable of producing and secreting antibody. A fraction of activated B lymphocytes do not differentiate into plasma cells, but rather form a pool of memory cells. The memory cells will respond to subsequent encounters with the pathogen, generating a quicker and more vigorous response to the pathogen. Some B lymphocytes can become activated without help from T lymphocytes, but these responses are generally weak and do not invoke memory.9,19

NK cells, often referred to as large granular lymphocytes, are defined functionally by their ability to lyse target cells without prior sensitization and without restriction by MHC. Resting NK cells express the intermediate-affinity IL-2 receptor, CD122. Upon exposure to IL-2, NK cells exhibit greater cytotoxic activity against a wide variety of tumors. NK cells recognize target cells by two mechanisms. First, NK cells express an IgG Fc receptor, CD16 that allows recognition of IgG-coated cells. Second, NK cells express killer-activating and killer-inhibiting receptors. The killer-activating receptors recognize multiple targets on normal cells; however, the binding of MHC class I to the killer-inhibitor receptor blocks release of perforins and granzymes. Therefore, cells (e.g., tumor cells, virally infected cells) that downregulate MHC class I expression are susceptible to NK cell cytolysis. NK cells play important roles in the surveillance and the destruction of tumors and virally infected host cells, and in the regulation of hematopoiesis.9,35

The immune system employs several mechanisms to downregulate responses to prevent autoimmune diseases. Many of these mechanisms are directed at T-lymphocyte activation. After activation (about 2 days), T-lymphocytes express CTLA-4 (a second ligand for B7, CD152). CTLA-4 (CD152) binding to B7 produces a negative signal to downregulate T-lymphocytes. In inflamed tissues, another mechanism of downregulation predominates, the programmed cell death 1 (PD1) system. In this system of downregulation, the PD1 protein becomes expressed on T-lymphocytes after activation. Inflamed tissues upregulate two ligands for PD1, PD-L1 (programmed cell death 1 ligand; CD274) and PD-L2 (programmed cell death 1 ligand; CD275). Binding of either of these ligands to PD1 results in the downregulation of the T-lymphocytes.26 Another use of the Fas system in addition to a mechanism by which CD8+ T-lymphocytes destroy their targets, the Fas system can provide a mechanism for the downregulation of T-lymphocyte responses. After activation, T-lymphocytes upregulate the cell surface expression of both Fas (CD95) and the ligand for this molecule (FasL). Binding of FasL (either expressed on the surface of a cell or soluble) to CD95 on the T-lymphocyte induces apoptosis (cell death) of the T-lymphocyte. Certain tissues (testis, retina, and some tumors) use the Fas system to protect themselves from harmful immune responses. These tissues constitutively express the FasL, which protects them from activated T-lymphocytes.26 Finally, the last mechanism to downregulate T-lymphocytes responses does not involve surface proteins, but a functional subset of CD4+ lymphocytes, T-regs. Tregs are antigen specific and require contact between the Tregs and the target cells. Tregs downregulate T-lymphocyte responses by secreting transforming growth factor-β and IL-10.32

**Soluble Mediators of the Adaptive Immune Response**

When binding of a specific antigen to the surface immunoglobulin receptor of B lymphocytes occurs, the B lymphocyte matures into a plasma cell and produces large quantities of antibody that have the ability to bind to the inciting antigen. The secreted antibodies may be of five different isotypes. On primary exposure to the pathogen, the plasma cell will secrete IgM; then, eventually, there is a switch to predominately IgG. On second exposure, the memory
B lymphocytes will predominately produce IgG. Isotype switching from IgM to IgG, IgA, or IgE is controlled by T lymphocytes.

An antibody or immunoglobulin is a glycoprotein comprised of two different chains, heavy and light (eFig. 21-6). The basic structure of every immunoglobulin consists of four peptide chains: two identical heavy chains and two identical light chains held together by disulfide bonds. The basic structure of the antibody is a Y-shaped figure. Each arm of the Y is formed by the linkage of the end of the light chain to its heavy chain partner. These arms contain the variable (V) and constant (C) regions. A light chain consists of one variable (VL) and one constant (CL) region. Heavy chains consist of one variable (VH) and three or four constant (CH) regions, depending on the isotype. The variable regions (VH and VH) compose the antigen-binding region of the IgG molecule, or fragment antigen binding (Fab). The constant regions provide the structure to the IgG molecule as well as binding the first component of complement (C1q) and binding to Fc receptors via the Fc portion of the molecule (Cn).
Organ-Specific Function Tests and Drug-Induced Diseases

SECTION

Assessment of a patient’s immune function requires knowledge and understanding of multiple components including mechanical defenses, cell phenotypes and cell numbers, and soluble components. Recent developments in biotechnology have allowed for progress in further characterization of immune system components and their severe combined immunodeficiency or hypogammaglobulinemia) or acquired, as seen with HIV infection. Autoimmune diseases result from a dysregulation of a component or a combination of components of the immune system (e.g., rheumatoid arthritis, systemic lupus erythematosus [SLE]). Autoimmune diseases are often characterized by production of autoantibodies against a particular host structure that is critical for normal function, or loss of tolerance orergy to a ubiquitous antigen (i.e., gluten in celiac sprue). Often medications that suppress the immune system are necessary to control symptoms and halt autoimmune disease progression. Exposure to immunosuppressant medications in the setting of autoimmune diseases or organ transplantation may reduce disease symptoms but at the cost of the host’s ability to fight off infection or cancer. Exogenous regulation of the immune system must be done judiciously, and we must continue to discover new methods for the appropriate evaluation of immune responses.

EVALUATION OF IMMUNE FUNCTION

DISEASES OF THE IMMUNE SYSTEM

Although this chapter is not intended to detail the diseases of the immune system, it is necessary to review the terminology and provide specific examples of diseases of the immune system to understand the role of monitoring and possible intervention with pharmacotherapy. Diseases of the physical defense immune system are often not thought of as diseases of the immune system, but the loss of normal physical defenses is the most common cause of impaired immunity resulting in infectious sequelae. For example, thick respiratory secretions secondary to altered chloride transport in cystic fibrosis lead to pathogen airway colonization. Primary immunodeficiency diseases are those characterized by either a genetic inability to produce components of the immune system (i.e., complement, antibody, and cytokines) and cells (e.g., neutrophils, macrophages, DCs, T lymphocytes, and B lymphocytes) are needed. The innate system will usually respond first. DCs, macrophages, and neutrophils in the tissues will recognize pathogen via surface receptors (eFig. 21-2). In order to amplify the immune response, the APCs will present antigen to CD4⁺ T lymphocytes (eFigs. 21-3 and 21-4). The activated CD4⁺ T lymphocytes will then secrete cytokines to activate B lymphocytes, CD8⁺ T lymphocytes, NK cells, macrophages, and neutrophils. The next section of the chapter discusses the evaluation of the immune system.

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functions. This is important because the upregulation and down-regulation of immune responses is necessary to treat various disease states. Therefore, pharmacotherapeutic considerations must balance the risk of disrupting normal immunologic homeostasis. Improvements in immune monitoring are necessary for the goal of patient-specific immunologic pharmacotherapy. Despite the technological advances, careful patient evaluations are required to accurately assess the structure and function of the immune system. Specific methods for assessment of patient immune status are discussed below.

### Innate Immunity: Evaluation of Mechanical Immunodefenses

As discussed earlier, the mechanical aspects of host defense are extremely important in protection from infection; therefore, assessment of mechanical defenses is critical. Much of the assessment of mechanical immunodefense is accomplished by recognition of situations where such defense is compromised. Careful patient examination usually reveals the extent of compromise, and laboratory tests are generally not necessary for evaluation of this component. To assess the extent of compromise in mechanical immunodefenses, the clinician should carefully examine the patient and identify the specific types of risks present. Specific examples of altered mechanical defenses are listed in eTable 21-6.

### Innate and Adaptive Immunity: Gross Evaluation of Cellular Components

A major aspect of the assessment of immune function relates to the cells of the immune system. Assessment of cells in the clinical setting includes determination of cell type, cell number, and/or function. Generally, determination of the cell types and quantification of the cell numbers are performed first because of the ease of obtaining these results and the common correlation with the clinical situation.

To quickly screen cell numbers, a white blood cell (WBC) count with differential is performed. Normal cell counts are shown in eTable 21-7. This simple test often steers the differential diagnosis. In interpreting a WBC with differential, the clinician must consider several factors. A normal cell count does not mean that a leukocyte disorder does not exist. For example, in chronic granulomatous disease, a child has a normal neutrophil count, but the neutrophils are unable to destroy the bacteria. Second, a differential is reported as percentage of the white blood cell (WBC). Therefore, one must also assess the absolute number as well as the percentage of white cell subtypes. For example, a patient admitted to the hospital with pneumonia has an elevated WBC (15,000 cells/mm³ [15 × 10⁹/L]) with a manual differential of 70% segs, 10% bands, 15% lymphocytes, and 5% monocytes. The WBC is predominately neutrophils; segs or mature neutrophils (70%) and bands or immature neutrophils (10%). The percentage of lymphocytes appears low at 15% (eTable 21-7), but the absolute number of lymphocytes is actually normal, 2,250 cells/mm³ (15,000 cells/mm³ × 0.15). A third factor to consider is that the majority of lymphocytes are in secondary lymphoid organs (e.g., lymph nodes, spleen), and changes in peripheral blood lymphocytes do not always mirror changes in the secondary lymphoid organs. Additionally, the majority of granulocytes, macrophages, and mast cells are also in the tissues, not the bloodstream.

To assess the numbers of granulocytes (neutrophils, basophils, and eosinophils) and monocytes, one uses a WBC with differential. An increased WBC count with immature neutrophils (e.g., bands, metamyelocytes, myelocytes, and promyelocytes) in the peripheral blood is called a “shift to the left,” and is abnormal. It most often indicates a bacterial infection, but also can indicate trauma or leukemia. It has long been recognized that the lower the absolute neutrophil count, the greater the risk of infection. Drugs (e.g., chemotherapy) and diseases (e.g., collagen vascular disorders) may lower the neutrophil count and make the patient more susceptible to infections. Patients with a neutrophil count below 1,500 cells/mm³ (1.5 × 10⁹/L) are considered to have neutropenia. Functional analysis of these cell types is rarely done in routine clinical practice. Patients with suspected functional deficits in these cell types are generally referred to tertiary medical centers for evaluation and treatment.

A routine WBC with differential can determine the total lymphocyte count. Total lymphocyte count has been used as a measure of nutritional status, as this rapidly changes with nutrient loss or repletion. This is a relatively gross measure of a patient’s immune status although it has been correlated to patient outcome and risk of infection.

Lymphocyte populations with different functions or in various stages of activation can be enumerated based on their cell surface markers. These cell surface markers are known as clusters of differentiation (CD). The CD is usually a protein or glycoprotein on
the surface of the cell. CD followed by a number designates the marker. Hundreds of monoclonal antibodies have been designed to recognize these cell surface markers. Monoclonal antibodies can be labeled with a fluorescent marker. The labeled monoclonal antibodies are then incubated with the patient’s cells. The antibodies will recognize and bind to the cells expressing the CD of interest, and the cells are then counted using flow cytometry. For flow cytometry, the cell suspension is put under pressure such that the cells flow past a laser in a stream of single cells. The laser will excite the fluorescently labeled antibodies bound to the lymphocytes. A light detector is able to count the labeled cell as the fluorescent tag emits light and determines the size of the cell based on its light scatter. These evaluations are valuable for assessment of patients with immune deficiency states such as AIDS or leukemias, and for patients who have received organ transplants. For example, clinically quantification of CD3 and CD4 cells is used, for example, to monitor muromonab, a monoclonal antibody directed against the CD3 receptor. In addition, the number of CD4 cells in HIV-positive patients correlates with the risk of and opportunistic infection and delineates the time to initiate antiviral therapy. Some of the more common CD antigens and their respective cellular distribution are listed in Table 21-8. Flow cytometry can be used for leukocyte phenotyping, tumor cell phenotyping, as well as for some types of DNA analysis.

**Innate and Adaptive Immunity: Functional Evaluation**

Counting the number of cells may help you estimate the function of the immune system, but several disease states exist in which there is an adequate number of cells but they are nonfunctional or they do not produce cytokines to communicate effectively. No single test exists to predict the function of the immune system with 100% accuracy. However, available tests can measure the viability of certain cell lines and communication between cells. Historically, the most common in vivo assay of lymphocyte function is the delayed hypersensitivity skin test. This test specifically evaluates the presence of delayed-type hypersensitivity or the presence of memory T lymphocytes. Specifically, a small amount of antigen, of which the patient is known to have been previously exposed, is administered. Under normal immunologic host conditions, exposure to this amount of antigen in the skin should produce lymphocyte infiltrate into the area within a few hours; followed by additional lymphocyte recruitment and phagocytes (e.g., macrophages, neutrophils) translocation. The maximal intensity of the inflammatory reaction occurs by 24 to 72 hours. This reaction is often referred to as type IV hypersensitivity (i.e., cell mediated; eChap. 22). A delayed-type hypersensitivity reaction is a test of cell-mediated immunity used to assess immunocompetency. The most common method to assess delayed-type hypersensitivity is to administer intradermally a panel of recall antigens. Commonly used antigens include Candida albicans, mumps, trichophytin, tetanus toxoid, and purified protein derivative (PPD) of tuberculin. Measurements in millimeters of induration at the site of injection should be taken 48 to 72 hours after placement of the antigens. A reaction is considered positive if the diameter of induration is ≥2 mm or greater. The degree of sensitivity correlates to the area of induration. Reaction to even a single antigen indicates a functioning cell-mediated immunity. The majority of immunocompetent individuals will show a positive reaction to at least one of these antigens. Possible reasons for not mounting a response to these antigens include congenital T-lymphocyte deficiency, cancer, HIV, or immunosuppressive drug therapy. No response is sometimes mounted because the individual being tested has not been previously exposed to a particular test antigen, although this is rare.

Global assessment of the in vivo immunologic response is also used commonly in solid organ transplantation during the diagnosis and assessment of acute rejection. For example, cellular rejection is detected on gross tissue biopsy by counting the number of lymphocytes present in the tissue and correlating their presence with other clinical findings, such as increasing serum creatinine in kidney transplant.

In vivo assessment of B-lymphocyte function involves immunizing the patient with a protein (e.g., tetanus toxoid) and a polysaccharide (e.g., pneumococcal polysaccharide vaccine) antigen to elicit and measure antibody responses after immunization. Two to 3 weeks after immunization, the patient’s serum is tested for antibodies specific for the immunized antigen. This test measures B-lymphocyte responsiveness to the inoculated antigens but is reserved for patients who are suspected to have impaired B-lymphocyte function.

A number of specific in vitro lymphocyte functional assays are used in the research setting. A few assays are performed at specialized clinical laboratories. One of these tests is the lymphocyte proliferation assay. In this assay, lymphocytes are obtained from a patient’s peripheral blood and cultured in vitro. The cells are exposed to nonspecific mitogens such as pokeweed mitogen, phytohemagglutinin, or concanavalin A. Then the cells are incubated in growth media containing tritium-labeled (3H) thymidine, a nucleotide used in the synthesis of DNA. Normally in the presence of the mitogens, lymphocytes will be stimulated to proliferate. Proliferating lymphocytes will incorporate 3H thymidine as they replicate DNA. The level of radioactivity of the cells can be measured on a β-scintillation counter and is proportional to the degree of proliferation. The patient sample needs to be compared to normal, healthy controls’ lymphocytes. Patients with immune deficiencies (AIDS, cancer, etc.) have fewer active or less active lymphocytes, as detected by this test.

A modification of the lymphocyte proliferation assay can be used in allogeneic bone marrow transplantation to evaluate how closely a donor and host are “matched” (i.e., cell mediated; eChap. 22). A mixed lymphocyte culture (MLC) can be used to assess the potential of the donor cells to attack the host cells, graft-versus-host disease (Chap. 117). In this test, donor cells and host cells are incubated in vitro. The host lymphocytes are irradiated prior to the incubation so that they cannot proliferate. In vitro, 3H thymidine is provided to the cells and uptake is measured. The degree of uptake correlates to level of proliferation of donor lymphocytes. If the cells are well matched, proliferation is minimal. If the cells are mismatched, proliferation will be noted with the level of proliferation predictive of the potential extent of graft-versus-host disease. With the introduction of DNA-based testing, these reactions are no longer used in the clinical setting.
molecular typing of HLA antigens, the MLC is rarely used today. However, the MLC may play a role in selecting not completely histocompatible donors.46

The Cylex Immune Cell Function Assay was recently FDA approved as a novel test used to determine the magnitude of suppression of CD4+ cells.47 Briefly, activity of CD4+ cells is measured by quantification of the amount of ATP produced and characterized as high, medium, or low.48 Initial, retrospective experience has been reported in the solid organ transplant population. Although this assay is in its clinical infancy, it is one of the first functional assays aimed at assessing individual patient response to immunosuppressive therapy. This type of testing may allow for tailoring of immunosuppression.

More recently, evaluation of immune cell activity such as factor for hand box P3 (FOXP3) T-regulatory cell activity has been used to evaluate the incidence and severity of acute rejection based on elevated FOXP3 mRNA expression in urine and tissue cell samples. T-regulatory cells control autoimmune reactions; therefore, the presence of these cells in an allograft may indicate a level of “tolerance” to the donor tissue. Tolerance, basically, is a state in which the body knows that foreign tissue (e.g., kidney transplant) exists but does not attack it. Initial studies have evaluated biopsy samples from organ transplant recipient and correlated them with levels of FOXP3. Early evidence suggests that FOXP3 is present only during periods of inflammation, such as rejection, and potentially projects the allograft tissue.49 In addition to the tests described above, a number of other tests and assays have been devised to evaluate the function of CD8+ T lymphocytes, NK cells, and monocytes/macrophages. Although these evaluations are not commonly performed, they may be helpful in some specific diseases and will likely be the way in which we monitor and detect immunologic events in the future. A thorough discussion of these tests is available.50

**Evaluation of Cytokines and Chemokines**

Often disease states involving the loss or upregulation of cytokines and chemokines are overlooked as diseases of the immune system. However, as we have just reviewed, cytokines and chemokines are essential components of both the innate and adaptive immune systems and provide the communication linking them together. Assays of humoral components may be either quantitative to determine the absolute concentration of the factor or qualitative to determine the function of the component.

**Immunoglobulins**

Measurement of immunoglobulins is a direct measure of B-cell function. The most common evaluation of immunoglobulins is the estimation of total immunoglobulin. This is approximated by subtracting the albumin concentration from the total protein concentration in serum. This difference gives a gross estimation of the total immunoglobulin concentration. Actual determination of the total immunoglobulin concentration is done by SPEP. Five separate zones are detected by this method: albumin, α-globulin, α2-globulin, β-globulin, and γ-globulin.5

The γ-globulin fraction contains the five isotypes of immunoglobulin (i.e., IgG, IgA, IgM, IgE, and IgD). A normal total immunoglobulin or γ-globulin concentration ranges from 0.8 to 1.6 g/dL. This test is used to determine if patients have hypogammaglobulinemia (i.e., primary and secondary immunodeficiencies), a monoclonal peak (e.g., multiple myeloma, Waldenstrom macroglobulinemia), or a polyclonal hypergammaglobulinemia (e.g., chronic inflammatory conditions such as SLE or chronic active hepatitis). Total immunoglobulin or γ-globulin concentrations cannot be used to measure antigen-specific antibodies or specific isotypes, although other evaluations can.

In a patient suspected of having humoral immune deficiency or B-lymphocyte failure (i.e., primary and secondary hypogammaglobulinemia), specific immunoglobulin isotypes in the plasma should be measured.

There are many indications for the measurement of antigen-specific antibody. Some common indications are listed in eTable 21-9. Methods to perform these measurements include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and radioallergosorbent test (RAST) (eFig. 21-7). The most common reason to measure antigen-specific antibody is to determine whether or not a patient has been exposed to an infectious agent. Generally,
IgM antibodies directed against the pathogen indicate an active or recent infection while IgG antibodies directed against the pathogen indicate prior exposure. This observation correlates with our understanding of B-lymphocyte responses in which plasma cells produce IgM initially in response to an infection, but later switches to IgG. Therefore, IgM antibodies will be present during an active infection and shortly after recovery from the infection. IgG concentrations will increase at the end of the primary exposure, but predominate after a second exposure. IgG predominates after a second exposure because memory B lymphocytes predominately secrete IgG in the serum. Other uses of antigen-specific antibody include determining if a patient has had exposure and is likely to be protected from infection (e.g., hepatitis A virus) or to determine adequate response to vaccination (e.g., hepatitis B vaccine). Clinically, measurement of antihuman IgG antibodies is used pre- and post-solid organ transplant to detect and potentially predict allograft compatibility and treat antibody-mediated rejection.

Antigen-specific IgE is commonly measured in patients with allergies. Because the presence of antigen-specific IgE is related to clinical allergy, measurement of these antibodies can be helpful in diagnosing allergies and determining offending substances. A standard method for determination of allergen-specific IgE is the RAST. The basic technique involves adding the antigen of interest, which is bound to beads or disks, to the patient’s serum. After precipitation and several washings, the antibody bound to the bead or disk is isolated. Finally, a radiolabeled antibody that binds to IgE is added. After further washings, the radiolabeled antibody bound to IgE, which is bound to the antigen on the bead or disk, is counted on a gamma counter.

Antigen skin testing is the preferred method to determine the presence of allergen-specific IgE. When it is produced, IgE binds to high-affinity IgE Fc receptors on basophils or mast cells. Contact of an allergen with the specific IgE on the basophil or mast cell surface causes activation of these cells and the release of inflammatory mediators (e.g., histamine). When this occurs systemically, it can cause anaphylaxis. When it occurs in a confined area such as the skin, erythema and induration are observed within a few minutes of allergen injection. This is the principle used for detection of penicillin allergy as well as for environmental or food allergies. A positive skin reaction (≥5 mm of induration) within 15 to 20 minutes is indicative of the presence of allergen-specific IgE.

There are four subclasses of IgG: IgG1, IgG2, IgG3, and IgG4 that make up 65%, 20%, 10%, and 5% of total plasma IgG, respectively. Concentrations of the subclasses are often measured in patients with suspected primary and secondary hypogammaglobulinemia. IgG2 and IgG4 deficiencies are associated with chronic infections. IgG4 deficiencies are also associated with autoimmune disorders.

**Complement System**

The complement system consists of a group of over 30 different proteins involved in lysing and opsonizing invading pathogens as well as serving as chemotactic factors. Numbers following the letter C (e.g., C1, C2) name the various proteins of the complement system. A test for the global assessment of the complement system is the CH₅₀, the total hemolytic complement test. This test is based on the premise that complement is needed for a rabbit anti-sheep antibody to lyse sheep red blood cells. The source of the complement is the patient’s serum. Each laboratory standardizes the test so normal ranges vary, but a standard curve is developed by adding titrated amounts of sera and measuring the amount of hemolysis. The hemolysis is determined with a spectrophotometer to measure the amount of hemoglobin released. The patient’s serum is then tested, and the amount of serum that is needed to lyse 50% of the red blood cells is reported as the CH₅₀. This test does not provide an indication of the function of any specific complement component but is used as a screening test for any complement system defects. If a defect is found, individual complement proteins can then be evaluated by either functional or immunochemical methods. Assessment of the complement system is important in patients suspected of having humoral immune deficiencies (i.e., recurrent infections).

Several disease states can alter complement concentrations. Low complement concentrations are frequently found during states of acute inflammation (e.g., SLE, rheumatoid arthritis, collagen vascular disorders, poststreptococcal glomerulonephritis, and subacute bacterial endocarditis). These states of apparent low complement concentrations are generally due to high rates of complement utilization or consumption that cannot be compensated for by increased complement synthesis.

Since the liver is the primary source of several components of the complement system (i.e., C2, C3, C4, factors B and D), a global decrease in complement factors occurs in severe liver failure. Inherited complement deficiencies have been described in patients with SLE, autoimmune diseases, recurrent gonococcal and meningococcal infections, membranoproliferative glomerulonephritis, and hereditary angioedema.

Clinically, the complement system has been used to diagnose and treat solid organ rejection. Antibody-mediated or humoral rejection is evaluated by quantifying the amount of donor MHC-specific antibody present in the recipient’s serum. The presence of donor-specific antibodies is correlated with evidence of antibody-mediated rejection on tissue samples. This is characterized by the presence of complement split products, namely C4d, which is present after complement-dependent antibody-mediated rejection. C4d covalently binds to the allograft tissue and can be stained for biopsy samples. Unfortunately unless biopsy findings can be correlated with a clinical finding consistent with rejection, the presence of C4d and its prognosis on long-term allograft function are unknown.

**Cytokines**

Cytokines are an important means of communication among cells of the immune system and other organ systems. Multiple cytokines with overlapping and redundant functions have been identified. Methods to detect and measure cytokines in biological samples have been developed. For nearly all the currently identified cytokines, commercial kits are available to measure endogenous and exogenously administered cytokines. The most common and preferable methods to measure cytokines are ELISAs and RIAs. ELISAs and RIAs are easy to run and measure immunoreactivity but not biologic activity (eFig. 21-7). Bioassays measure biologic activity, but are cumbersome and extremely variable. Using ELISA, we are able to measure only how much cytokine was produced by the cells in the culture. An ELISPOT is an enzyme-linked assay for detecting and enumerating cytokine-producing leukocytes. In contrast to conventional ELISA, ELISPOT allows the user to detect absolute numbers and frequencies of cytokine-secreting leukocytes.

We are still at the very early stages of interpreting the clinical relevance of endogenous cytokine concentrations. Not only is the immune system affected by cytokines such as IL-1, IL-6, tumor necrosis factor-α (TNF-α), but other systems (skeletal, endocrine, CNS) are also affected. Therefore, measurement of cytokine concentrations may be important in the evaluation of other systems as well as the immune system. Administering cytokines in clinical practice may change not only the concentration of that particular cytokine, but also the resultant concentration of other cytokines. For example, systemic administration of GM-CSF to patients not only increases concentrations of GM-CSF but also of TNF-α, IL-6, IL-8, macrophage colony-stimulating factor, and EPO. Secondary endogenous cytokine release should be taken into account when considering the
therapeutic effects of these agents and when monitoring cytokine concentrations.

In the future, tissue concentrations as well as blood concentrations may be measured. For example, while many centers currently measure cyclosporine concentrations to estimate the potential for immunosuppressive effects, it may be advantageous to monitor IL-2 concentrations. One of the primary actions of cyclosporine is the inhibition of IL-2 production. Furthermore, perhaps it would be beneficial to measure tissue concentrations of IL-2 in the transplanted organ to get a better estimate of the extent of immunologic suppression.

**Soluble Receptors and Receptor Antagonists**

The inflammatory response is highly regulated. The activity of cytokines, their receptors, and their antagonists are in a delicate balance. Although cytokine receptors are typically thought of as being found on the target cell, soluble cytokine receptors can modulate the activity of cytokines in at least two ways: (a) acting as antiinflammatory agents by binding cytokines with high affinity, but without biological activity;66 (b) augmenting cytokine activity by prolonging the cytokines plasma half-life and even maintaining agonist activity on cells that do not inherently respond to the cytokine.67 Finally, antagonists to cytokine receptors have been identified.

Tumor necrosis factor-α plays a central role in the inflammatory response by both increasing the expression of adhesion molecules in the tissues and by stimulating production of proinflammatory cytokines (e.g., IL-2, IL-8), prostaglandins, and nitric oxide. Soluble tumor necrosis factor receptors (sTNFRs) act primarily as inhibitors of TNF by preventing TNF from binding to the membrane-bound TNFRs, or by causing the cells to shed the receptor from the surface of the cell so that it can no longer serve as a signaling molecule.68 Both monoclonal antibodies against TNF (e.g., infliximab, adalimumab) and sTNFRs (e.g., etanercept) have been shown to modulate the activity of TNF and are used clinically for the treatment of autoimmune diseases.

The best characterized receptor-binding antagonist is the interleukin-1 receptor antagonist (IL-1RA). IL-1RA blocks the binding of IL-1 to its receptor by competing for the same binding site, but IL-1RA does not possess agonist activity.69 A recombinant IL-1RA, anakinra, is used clinically for the treatment of severe rheumatoid arthritis.69

Our developing understanding of soluble receptors and receptor antagonists allows us to better mimic natural mechanisms for minimizing the toxicity of exogenously administered cytokines (e.g., IL-1, IL-2, TNF-α, etc.) as well as immunomodulate various diseases (e.g., solid organ transplant rejection, collagen vascular disorders, sepsis, etc.).

**Modulation of the Immune Response**

Modulation of the immune response through administration of pharmacological agents or with blood product components does not come without a risk gained with the benefit. Providing supplementation to the immune system, for example during periods of sepsis with recombinant activated protein C (drotrecogin alfa), provides increased levels of protein C which decrease levels of proinflammatory IL-6 potentially limiting the effects of the immune reaction and preventing end organ damage at the cost of bleeding for over exposure to protein C. Consequently, this agent has been removed from the market after no mortality benefit was seen. While TNF inhibitors interfering with the immune system to halt the damage of an autoimmune disorder may suppress symptoms, it places the patient at risk for opportunistic viral infections. Many of our newer biological agents directed at a pathway in the immune system are genetically engineered from animals and humanized to increase their biological effectiveness. As a result, these agents can serve as antigens to the immune system and display variable effectiveness between and among patients over time. For example, an agent commonly used in solid organ transplantation is rabbit antithymocyte globulin, which is a polyclonal antibody directed against lymphocytes which given at the time of transplant or to treat rejection. Patients with previous exposure to this agent may have developed antibodies against the rabbit epitope of the drug. This results in either a decrease in the effectiveness of the drug, because it is bound by antibody or production of an immunologic reaction, commonly manifested by antibody–antigen complex deposition in the kidneys and joints producing high fevers and renal failure. Based on the very few examples presented here, one can understand why manipulation of the immune system must be carefully assessed and appropriate patient instruction given.

**Immunosuppression**

Immunosuppression was first developed and used to allow transplantation of foreign tissues or to treat malignancies of the immune system. Today, the number of compounds and disease states in which the immune system is implicated is virtually unquantifiable. Therefore a thorough review of these drugs is beyond the scope of this chapter. These medications are usually very expensive and associated with potentially serious adverse effects. Immunosuppressants block critical steps of the immune response, and patients must be counseled on their risk of infection and the plan to monitor effectiveness of the immunosuppressant. Several key concepts and questions can be used to help clinicians discern the potential benefits and ramifications of administering any immunosuppressant. These include: (a) what is its mechanism of action, (b) what arm of the immune system does it effect, (c) when is its onset of action, (d) how was this compound derived and does it have the potential to stimulate antibody production if the patient is re-exposed, (e) is this compound’s effect dose or duration related, (f) what type of infection is my patient at risk for and do I need to administer prophylactic medications, and (g) how do I monitor the biological effect of this compound?

**Immunopotentiation**

In an attempt to restore normal immune system function or to activate the immune system, immunopotentiators are often used. The best example of immunopotentiation of the immune system is the practice of immunizations. Active immunization with a vaccine or toxoid induces the host’s immune system to confer protection against a pathogen (e.g., hepatitis A, hepatitis B, diphtheria toxoid, etc.). This process requires the uptake of the immunogenic epitope by APCs followed by presentation to CD4+ T lymphocytes and the subsequent development of either a cellular or humoral immune response.

In contrast to active immunization, passive immunity entails the administration of human immunoglobulin to provide short-term protection to individuals who will be or have been exposed to a pathogen. IV immunoglobulin (IVIG) consists of >90% polyclonal IgG that is prepared from donated plasma. In patients with primary or secondary hypogammaglobulinemia, IVIG restores circulating IgG concentrations thus decreasing the incidence of infections in these patients. In addition to restoring IgG concentrations, IVIG can potentially immunomodulate the immune response. For example in immune thrombocytopenic purpura, an autoantibody directed against the platelets leads to the destruction of the platelets...
by antibody-dependent cellular cytotoxicity. IVIG saturates the Fc receptors on phagocytic cells, thereby preventing the engulfment of autoantibody-opsonized platelets. IVIG can also contain antiidiotype antibodies to immunomodulate an immune response. Antiidiotype antibodies are directed against the idiotype or hypervariable region of a native antibody. After administration of IVIG, the antiidiotypes bind to the hypervariable region of the autoantibody and prevent the autoantibody from opsonizing circulating platelets. In addition, the antiidiotypes directed against the autoantibody can bind to the surface immunoglobulin on the B lymphocyte producing the autoantibody that leads to the destruction of the B lymphocyte.69

Through our increased understanding of critical steps in downregulating T-lymphocytes, two monoclonal antibodies that enhance T-lymphocyte activity have been developed for the treatment of cancer. Ipilimumab, a humanized monoclonal antibody, binds to CTLA-4 on activated T-lymphocytes. By blocking CTLA-4, T-lymphocytes remain active to attack their target. Single agent ipilimumab significantly improves overall survival in patients with unresectable or metastatic melanoma.60 However, one concern with ipilimumab and nivolumab resulted in significant activity in advanced melanoma.61 However, in the context of ipilimumab and nivolumab is the development of immune-mediated adverse events such as colitis, hepatitis, dermatitis, pneumonitis, and endocrinopathies.60,62

Sipuleucel-T immunotherapy shows significant activity in castration-resistant prostate cancer. Sipuleucel-T takes advantage of our increased knowledge of APCs. Sipuleucel-T involves isolation of APCs from a patient by leukapheresis followed by in vitro activation of the APCs by a recombinant fusion protein, PA2024, which contains GM-CSF to activate the APCs and a tumor antigen, prostatic acid phosphatase (PAP), common to prostate cancer. Finally, the activated APCs are re-infused into the patient every 2 weeks. The activated APCs then present PAP to the patient’s T-lymphocytes, which then leads to increased numbers of T-lymphocytes attacking the prostate cancer.63

### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>APC</td>
<td>antigen-presenting cell</td>
</tr>
<tr>
<td>CD</td>
<td>clusters of differentiation</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSF</td>
<td>colony-stimulating factor</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cell</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>EPO</td>
<td>erythropoietin</td>
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<tr>
<td>FasL</td>
<td>Fas ligand</td>
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<tr>
<td>Fc</td>
<td>fragment crystallizable</td>
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<tr>
<td>FOXP3</td>
<td>factor forkhead box P3</td>
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<tr>
<td>GM-CSF</td>
<td>granulocyte-macrophage colony-stimulating factor</td>
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<tr>
<td>IgG</td>
<td>immunoglobulin G</td>
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<tr>
<td>IL-1RA</td>
<td>interleukin-1 receptor antagonist</td>
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<tr>
<td>IVIG</td>
<td>IV immunoglobulin</td>
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<tr>
<td>MALT</td>
<td>mucosa-associated lymphoid tissue</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
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<tr>
<td>MIP</td>
<td>macrophage inflammatory protein</td>
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<tr>
<td>NK</td>
<td>natural killer</td>
</tr>
<tr>
<td>PD1</td>
<td>programmed cell death 1</td>
</tr>
<tr>
<td>PMN</td>
<td>polymorphonuclear cell</td>
</tr>
<tr>
<td>RIA</td>
<td>radioimmunoassay</td>
</tr>
<tr>
<td>SLE</td>
<td>systemic lupus erythematosus</td>
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<tr>
<td>SPEP</td>
<td>serum protein electrophoresis</td>
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<tr>
<td>TCR</td>
<td>T-cell receptor</td>
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<tr>
<td>TH1</td>
<td>T-helper type 1</td>
</tr>
<tr>
<td>TH2</td>
<td>T-helper type 2</td>
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<tr>
<td>TNF-α</td>
<td>tumor necrosis factor-α</td>
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<tr>
<td>WBC</td>
<td>white blood cell</td>
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### REFERENCES
