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The Immune System

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CHAPTER 2 | The Immune System

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Modern biotherapy has been in use for some 30 years. The first types of biotherapy were nonspecific stimulators of the immune response, but advances in genetic engineering are allowing the mass production of pure biological products which are now being tested as pharmaceutical agents. Biotherapy connotes the administration of products (1) that are coded by the mammalian genome; (2) that modify the expression of mammalian genes; or (3) that stimulate the immune system. In this chapter the discussion of the immune system will be limited primarily to topics relevant to cancer or autoimmune diseases.

Because understanding the new biological agents requires an understanding of both the immune response and the molecular basis of oncogenesis, this chapter first presents a summary of the structure and function of the immune system. Following a discussion of immune responses, and the cells involved in these responses, will be a discussion on the current concepts of oncogenesis, particularly oncogenes and growth factors. Because research efforts are beginning to identify many biological proteins as having a role in autoimmune and other diseases, a brief introduction to autoimmune diseases is also included at the end of the chapter.

THE IMMUNE SYSTEM

Overview of the Immune Response

The immune system is a complex, dynamic system which evolved to protect the individual against pathogenic organisms (Figure 2.1). In addition to recognizing and destroying foreign substances or **antigens**, immune responses can also destroy altered and malignant cells. This latter function of the immune system is called **immune surveillance.** The destruction of microorganisms and the de-

Figure 2.1   The humoral branch and cell-mediated branches of the immune system. The humoral response involves interaction of B lymphocytes with antigen and their differentiation into antibody-secreting plasma cells. The secreted antibody binds to the antigen and facilitates its clearance from the body. The cell-mediated response involves various subpopulations of T lymphocytes that recognize antigen presented on self-cells. T_H-cells respond to antigen with the production of lymphokines and cytotoxic T lymphocytes (CTL) mediate killing of cells that have been altered by antigen (e.g., virus-infected cells).
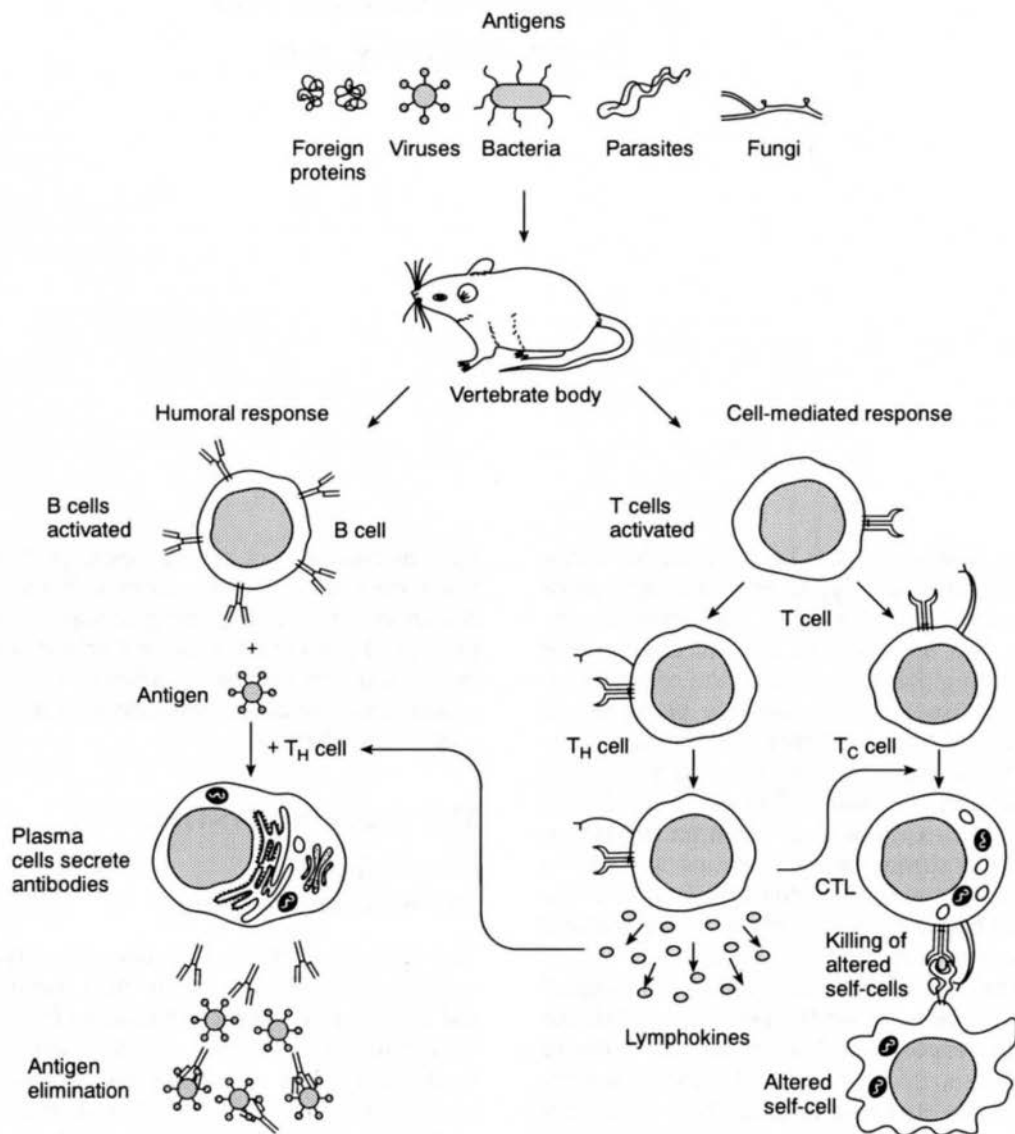
Table 2.1 Functions of the Immune System

Protection	Recognition and destruction of pathogenic microorganisms
Surveillance	Destruction of malignant cells
Homeostasis	Destruction of worn and damaged cells
Tolerance	Recognition of self components
Regulation	Augmentation and depression of immune responses

struction of tumor cells involve the same immunologic mechanisms. The mechanisms used to fight viral diseases are particularly important in immune responses to tumors. See Table 2.1 for a summary of functions of the immune system.

Immune responses involve a number of different cell types and their products. Because it is impossible to discuss or study all the responses at the same time, they are divided into two types, innate and adaptive. **Innate immunity** is also called natural or nonspecific immunity and includes the inflammatory response. **Adaptive immunity** is also known as specific or acquired immunity. For a review of innate and adaptive immune responses, see Table 2.2.

Innate immunity is the first line of defense against pathogenic organisms; adaptive immunity involves the recognition of specific foreign determinants and a memory response. To recognize and destroy what is foreign also implies that the immune system must be able to tolerate self, that is, to tolerate its own cells and their products. If the molecules on the microorganism or tumor cell are recognized as foreign (nonself or altered self) and stimulate a specific immune response, they are called antigens. The small area of the antigen that binds to the lymphocyte receptor is the **antigenic determinant** or **epitope.**

The cells and products of innate and adaptive immune responses act in concert and are all responsible for resistance to pathogens and surveillance. For instance, the first line of defense against pathogenic organisms (e.g. *Pseudomonas)* are mechanical barriers such as intact skin and mucous membranes. If these

physical barriers are breached, an inflammatory response attempts to limit the colonization of the microbe. The acute inflammatory response involves the physiological processes of capillary dilatation, exudation of fluid and plasma protein into the tissues, and accumulation of neutrophils at the site of infection. These responses are manifested clinically as the five cardinal signs of inflammation: heat, redness, swelling, pain, and loss of function. The invading organism is then killed through **phagocytosis** by neutrophils or soluble chemical factors generated during the inflammatory response (Figure 2.2). The influx of neutrophils is replaced by monocytes, which continue to migrate to the inflamed area. The activation of monocytes is the bridge between innate and adaptive immunity. Monocytes are capable of processing the antigen they phagocytize and presenting it to other white blood cells involved in adaptive or specific immunity.

Thus, if this innate response fails and the organism colonizes the body, the adaptive immune system is stimulated and responds. The specific immune response on the next exposure to the same antigen will be heightened and more rapid. This secondary response is called **memory** or **anamnestic response.**

Generally speaking, the white blood cells are responsible for immune responses; granulocytes (neutrophils, eosinophils, and basophils), monocytes and their tissue counterparts macrophages are involved in innate immunity, whereas lymphocytes are responsible for specific immune responses. Lymphocytes have two different ways of recognizing antigenic determinants. The first is by antibodies located on the membrane of B lymphocytes and the second is by T-lymphocyte (or T-cell) receptors. For the T-cell receptors to recognize the antigenic determinants the antigen must be present along with self molecules or **major histocompatibility (MHC) molecules.** Typically, both the innate and adaptive systems (both B and T lymphocytes) are needed to effect a response sufficient to contain and eliminate pathogens.

Table 2.2 Features of innate and adaptive immunity

| System	Hallmarks	Features and Functions

Innate Immunity	Primary line of defense
	Non-specific
	·No memory
		Mechanical Barriers
		  Intact Skin
		  Mucous Membranes
		Chemical Barriers
		Inflammatory response
		Fever
		Phagocytic Cells
		Soluble Factors
		Protects against pathogens

Adaptive Immunity	Secondary line of defense
	Specificity
	Memory
		Lymphocytes
		  T-cells: Provide cell mediated
		  immunity
		Primarily protects against intracellular organisms, immune surveillance, responsible for rejection of transplanted organs and modulation of immune response
		  B-cells: Provide humoral immunity
		Primarily protects against viruses and bacteria

ANTIGENS

The major function of the immune system is to distinguish between self molecules and non-self or altered-self molecules. Molecules that induce a specific immune response are called **immunogens.** All immunogens are also antigens. Allergens are antigens that induce allergic responses. The epitope or antigenic determinant is that specific part of an antigen, allergen, or immunogen that binds directly with the immunoglobulin or T-cell receptor. For example, an antigenic protein may consist of hundreds of amino acids, but only six or seven of these bind directly with an immunoglobulin. The binding area of the antigen is the epitope. **Tolergens** are molecules that induce a state of immunologic unresponsiveness. Most times antigens are exogenous molecules such as bacterial or viral proteins. Rarely, immune responses are generated to self molecules, that is autoantigens or autologous antigens, resulting in serious pathologic states called autoimmune diseases. **Alloantigens** are endogenous molecules that

distinguish one individual from another individual of the same species. The A, B, and O blood-type molecules and the histocompatibility antigens are examples of alloantigens. A bone marrow transplant between siblings is called an allogeneic transplant.

The distinction between self molecules and foreign molecules is a learned response. Exposure of the immune system to molecules *in utero* will generate tolerance to these molecules: the immune system will not respond to these molecules, rather they will be considered self molecules (self antigens). The greater the difference between self molecules and exogenous or foreign molecules, the more likely it is that an immune response will be generated. Also, the greater the evolutionary divergence between two species, the greater the likelihood that an immune reaction will occur. In humans, for instance, an immune response to avian albumin is more likely than a response to primate albumin. There are, however, some proteins that are phylogenetically

Figure 2.2   The inflammatory response. A bacterial infection causes tissue damage with release of various vasoactive and chemotactic factors. These factors induce increased blood flow to the area, increased capillary permeability, and the influx of white blood cells, including phagocytes and lymphocytes, from the blood into the tissues. The serum proteins contained in the exudate have anti-bacterial properties and the phagocyte will begin to engulf the bacteria.
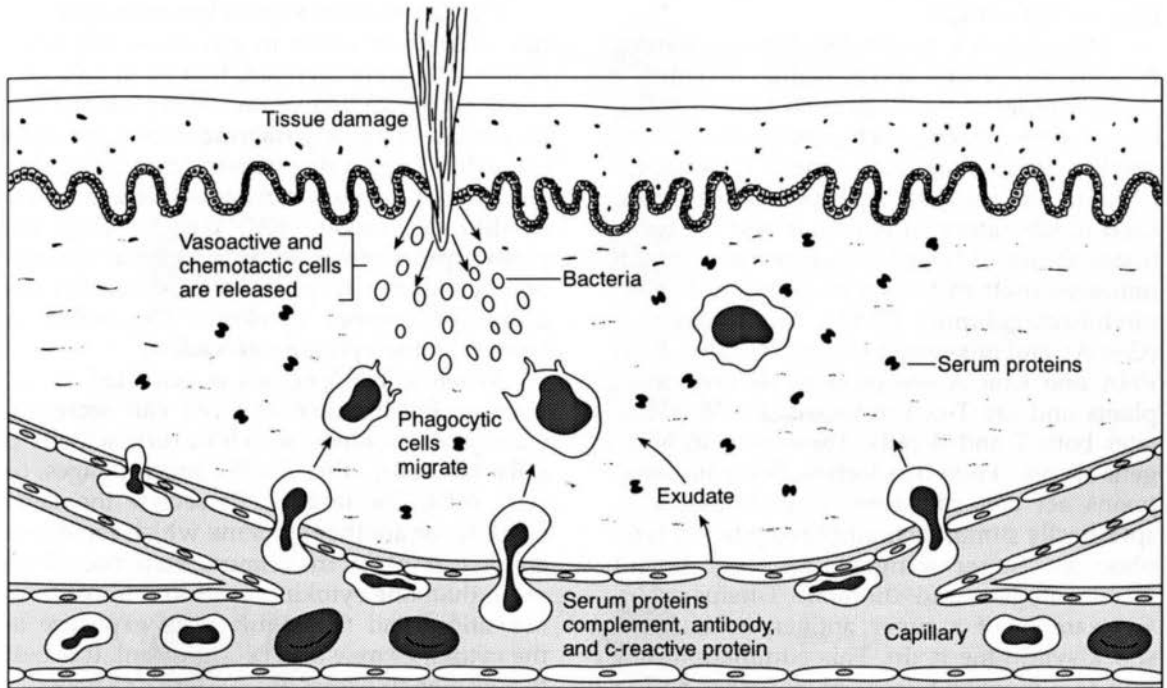
Source: IMMUNOLOGY by Janis Kuby. Copyright (c) 1992 by W.H. Freeman and Company. Reprinted with permission.

conserved, such as collagen; these proteins will not generate immune responses even if transplanted across species lines.

Besides the degree of foreignness, the size and the chemical nature of the molecules will determine if a specific immune response is generated. In general, proteins are more efficient immunogens than other compounds; complex carbohydrates are also good immunogens. Lipids and nucleic acids are poor immunogens unless they are bound to proteins. A complex molecule is likely to generate an immune response, whereas polymers composed of a single amino acid are unlikely to generate a response. The B and T lymphocytes

will recognize different epitopes on the same molecule. For the T cell to recognize a molecule as foreign, the molecule first must be phagocytized and processed, and then small segments of the molecule must be linked to MHC molecules on the surface of the accessory cell. Molecules that resist digestion by phagocytes are less likely to stimulate a T-helper cell response. If the T-helper response is limited, then neither a B-cell response nor a T-cell response is likely. The route by which the antigen is presented to the immune system and the dosage will affect the type and degree of immune responses. Antigens present in the mucosa are much more

likely to generate immunoglobulin A or E than immunoglobulin G. A systemically administered antigen is likely to produce an immunoglobulin G response. If the antigen is administered in very high quantities or in very low quantities, the host may come to tolerate the antigen.

Mitogens are molecules that can induce nonspecific division and activation of lymphocytes; mitogens will also stimulate many clones of lymphocytes. This contrasts with the specific stimulation of a single lymphocyte clone by an antigenic epitope. Mitogens are used in laboratory situations to test the functional ability of lymphocytes. Some common mitogens used in testing of lymphocytes are phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM). Both PHA and Con A are proteins derived from plants and are T-cell mitogens; PWM stimulates both T and B cells. These protein mitogens are also known as **lectins.** Some bacterial toxins act like mitogens in that they nonspecifically stimulate multiple colonies of lymphocytes. **Super antigens** are very potent T-cell mitogens and stimulate T-helper cells, an example of a super antigen is the toxic shock syndrome toxin. This stimulation may lead to a massive release of cytokines and to shock and death. The lipopolysaccharide in bacterial endotoxin is another example of a super antigen.

CYTOKINES

Cytokines and lymphokines are discussed here because these molecules are a part of a network of mediators released in inflammatory and specific immune responses. These compounds are produced primarily by T cells, but they can be produced by other types of cells such as monocytes, or their mature counterpart, macrophages. The term *cytokine* describes a soluble protein produced by cells, and includes polypeptide molecules such as interleukins, lymphokines, monokines, interferons, tumor necrosis factor, and transforming growth factor. They are biologically very

active, have short half lives, and are functional at low concentrations. Cytokines bind to plasma membrane receptors; they affect the growth and differentiation of white blood cells, and regulate immune and inflammatory responses.

Cytokines act as signals between cells and can exert their effect in the same cell from which they were secreted, that is, in an **autocrine** action. Cytokines can affect cells in the immediate area, a **paracrine** action, or they can exhibit **endocrine** actions, that is, on distant cells. A cytokine can have different effects in different target cells, that is, they are **pleiotropic.** Cytokines can also act in concert: one cytokine may potentiate (or inhibit) the action of another cytokine. This effect is known as the **cytokine cascade.**

When a T-helper cell is activated by an antigen, for instance, the cell can secrete a variety of cytokines, which in turn activate B cells, NK cells, other T cells, macrophages, or stem cells. The target cells secrete the same cytokine or another cytokine which can either amplify or inhibit the immune response. Often more than one cytokine is needed for cellular activation, and the sequence of exposure to the cytokines may also be important. It is best to consider cytokines as a system or a network in which the cytokines can act in a **redundant, synergistic, antagonistic** or **pleiotropic** fashion. (See Figure 2.3.)

When cytokines were first studied, their chemical structures were not known, so functional assays were developed for each one. Each cytokine was studied and named by the action it produced. For instance, investigators who studied a substance that destroyed tumor cells named it tumor necrosis factor (or lymphotoxin). Other investigators who studied a substance that caused weight loss called it cachectin. When the chemical natures of these compounds were described, it was discovered that tumor necrosis factor, lymphotoxin, and cachectin were really the same compound. More than one name exists for many of the cytokines. In 1986 an international system was established to try to stan-

Figure 2.3   Examples of the cytokine attributes of pleiotropy, redundancy, synergism, and antagonism.
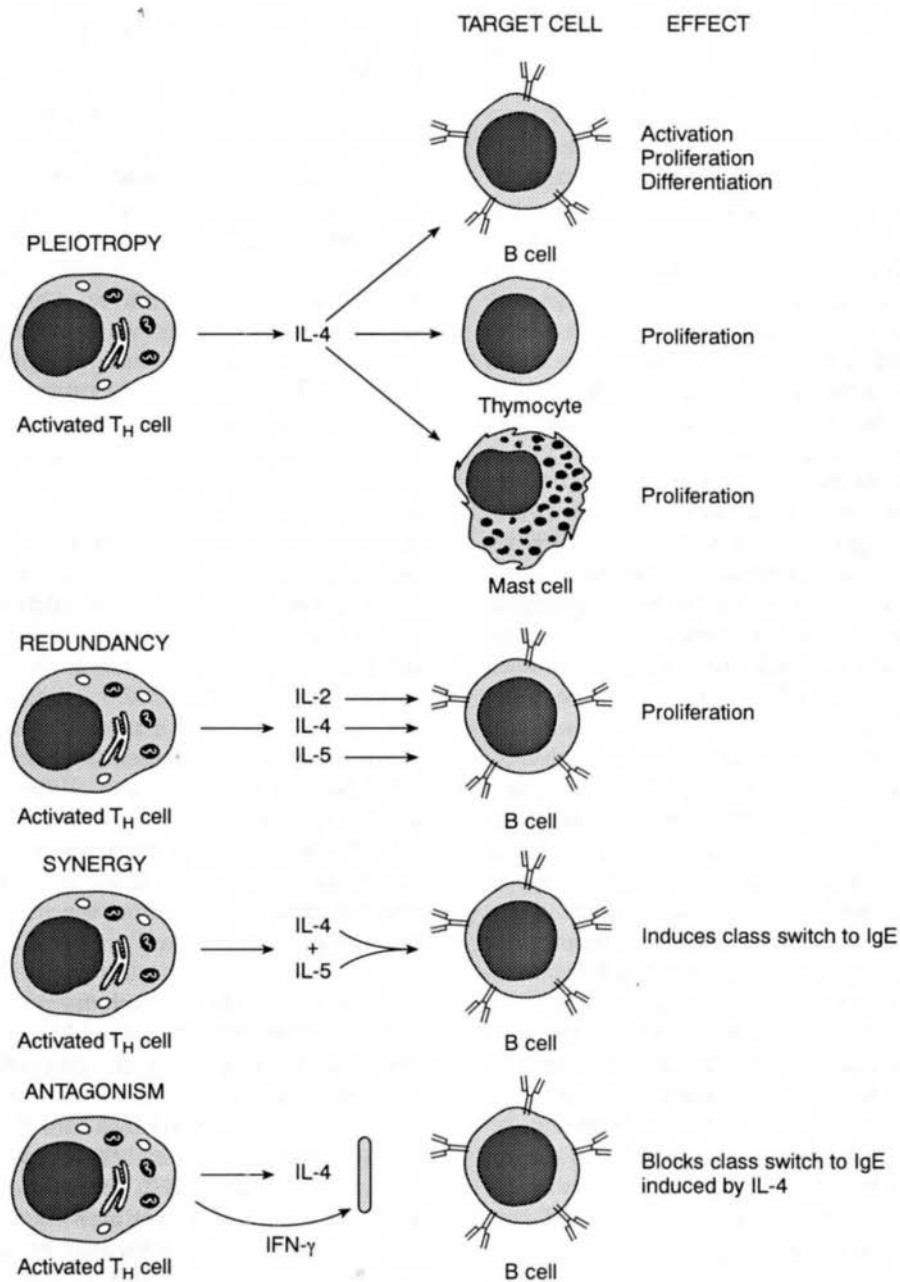
dardize the cytokine nomenclature. (See Chapter 5.) When the DNA sequence coding for a protein has been discovered, it is now given an interleukin designation. The term **interleukin** literally means "between white cells," however, some of these protein molecules are made by, or act on, cells other than white blood cells.

An example is interleukin–6 (See chapter 16). Previously it was called B-cell differentiation factor, B-cell stimulation factor, hepatocyte-stimulating factor and interferon-β2, depending on the assay used. Interleukin–6 stimulates T-cell proliferation and differentiation in addition to the production of IL–2 and IL–2 receptors by the T-cell. Interleukin–6 also stimulates plasma cells to secrete immunoglobulins and hepatocytes to secrete acute phase response proteins such as C reactive protein. Interleukin–6 is also important in hematopoiesis. It is produced not only by T-helper cells but also by macrophages and fibroblasts. Therefore, it acts in a pleiotropic, redundant, and synergistic fashion. Since it can also be manufactured by and influence cells other than white blood cells, the term cytokine may be better than interleukin.

Cytokines may also play a role in bidirectional communication between the neuroendocrine and immune systems. For example, interferon-α can increase the levels of circulating cortisol. Interleukin–2 infused in pharmacologic doses can cause fatigue and depression. Some of the cytokines induce fever by affecting the temperature-regulating center in the hypothalamus. Thus, cytokines may be important for integrating the immune response within the total person, not just within the immune system. Likewise, changes in the neuroendocrine system can heighten or diminish immune responses. Hormones such as cortisol and the sex hormones generally depress the immune responses, whereas growth hormone and thymosin enhance T-cell responses. This communication between systems is possible because there are receptors on the surfaces of lymphocytes and other white blood cells for hormones such as cortisol and epinephrine, while the nervous system has receptors for cytokines such as interferon-α and IL–2.

MAJOR HISTOCOMPATIBILITY COMPLEX

Regulation of immune responses occurs at many levels: by antigens, cytokines (between cells), and the neuroendocrine system. The ability to respond to an antigen is determined not only by the factors listed above but also by the genetic constitution of the individual. The part of the genome that we know the most about with respect to immune responses is called the **major histocompatibility complex (MHC)**. This area was first studied in relationship to transplantation of tissue. Because the first cells used for testing the compatibility of tissues between individuals were lymphocytes, in humans the MHC is called the **human leukocyte antigen (HLA) complex.** Here the term antigen is used, because lymphocytes from one person or donor, would be foreign to another person, or recipient, and would stimulate an immune response to the donor cells in the recipient. Much of the research done in this area has used rodent models. In the mouse, the MHC is called the H2 complex (H for histocompatibility and 2 for the second erythrocyte antigen of the mouse).

The MHC or HLA genes are located on chromosome 6 and code for molecules on the cell membrane needed for the presentation of the antigen to T lymphocytes. These genes also code for some soluble molecules, including those of the complement system and tumor necrosis factor. The MHC comprises about 40 to 50 genes and, although the functions of many are understood, others are not. The HLA is further divided into class I, II, and III regions; the genes code for class I, II, and III molecules. These MHC molecules are often referred to as MHC antigens.

Class I genes are located at the HLA-A, HLA-B, HLA-C, -E, -F and -G loci. The **locus** is the position on the chromosome where a gene is positioned. The HLA genes are located on the short arm of chromosome 6. The class I

genes code for molecules that are present on most nucleated cells. For a cytotoxic T cell to recognize an antigen, the antigen must be complexed with a MHC class I molecule. Considering that a virus can infect any cell in the body and that the cytotoxic T cell is responsible for killing cells infected with viruses, it makes sense that class I molecules are ubiquitous. Class I molecules are absent in sperm and in trophoblasts. Some cells of the body, such as liver cells, fibroblasts, and neurons, express very low levels of these genes. Perhaps the reason for the success of liver transplantation and the acceptance of the fetal tissues as grafts is the low numbers of class I molecules on the membranes of these cells. Cytokines such as tumor necrosis factor and interferons can increase (up-regulate) the expression of these genes, increasing the numbers of class I molecules on the surfaces of these cells.

Class II molecules are found on cells of the immune system: **B lymphocytes,** antigen-presenting cells, accessory cells, **macrophages,** Langerhans cells, and dendritic cells. The major role of class II molecules involves the presentation of the antigen to T-helper cells. The antigen is first processed, then it binds to the class II molecule; it is this complex that binds to and stimulates the T-helper cell. The class II genes are located at the DO, DP, DQ, DR, DV, DX, and DZ loci. The class II genes are also known as the immune response genes and determine the level of response to an antigen. Certain animal strains that tend to respond quantitatively more to an antigen than other strains are called high responders. The existence of high and low responder strains is due to differences in the MHC class II genes. Because both class I and II molecules have some structural similarity to the immunoglobulin molecules, they are considered to belong to the immunoglobulin superfamily.

Class III genes code for some of the proteins involved in the complement pathway, tumor necrosis factor, and steroid hydroxylase enzymes. These molecules are soluble, diverse, and have no role in presentation of the antigen. The class III genes lie between the class II and class I genes on the chromosome.

Haplotypes

There are many variants or **alleles** of each of the MHC genes, allowing many variants in each of the class I and class II molecules. This leads to the great diversity seen in humans and accounts for the difficulties in transplantation among unrelated donors. The MHC genes are tightly linked. Usually, one copy of the entire MHC gene (a **haplotype**) is inherited as a unit from the mother and another copy is inherited from the father. Twenty-three different alleles are known for the HLA-A locus of MHC class I, 49 for the HLA-B locus, and eight for the HLA-C locus. If there are 50 genes in the MHC and there are many variants of these genes; it is easy to see why it is difficult to match for histocompatibility. Theoretically, the number of possible MHC genotypes in the human population would be the number of alleles for each gene multiplied by every other possibility, i.e. 23(A) × 49(B) × 8(C) and so on for the 50 genes. In actuality, the total number of possibilities is less than the theory predicts because certain of these genes are tightly linked to each other making their recombination with other genes highly unlikely. Certain haplotypes are found more frequently than would be expected, so that in isolated populations a greater frequency of certain alleles of the MHC will be found. Because of the tight linkage, there is one chance in four that siblings will share the same haplotypes. In an individual cell both the maternal and paternal genes are expressed, that is, the genes are codominant.

TISSUES AND CELLS OF THE IMMUNE SYSTEM

The Lymphoid System

The cells responsible for the immune response are organized into tissues and organs collectively known as the lymphoid system. Lym-

phoid tissue can be designated as primary or secondary. The primary organs include the bone marrow and the thymus, and are the sites where lymphocytes develop and mature. Secondary lymphoid organs include the lymph nodes, lymph vessels, spleen, tonsils, and unencapsulated lymphoid tissue lining the respiratory, alimentary and genito-urinary tract. Lymphocytes are stored and activated in the secondary organs. Lymph nodes are chains of encapsulated lymphoid tissue housing B- and T-lymphocytes. They are found throughout the body and are located primarily at the junctions of lymphatic vessels, a network that drains and filters tissue fluid. In sum, lymph nodes filter material draining from body tissues, the spleen filters antigens in the blood, and the unencapsulated lymph tissue monitor mucosal surfaces.

Cells of the Immune System

The white blood cells (granulocytes, monocytes, lymphocytes) responsible for immune responses are derived from bone marrow stem cells in children and adults and the yolk sack, spleen, and liver in the fetus. The stem cells are pluripotent, that is, capable of producing all the formed elements of the blood, including erythrocytes (red blood cells) and platelets. This process of differentiation or maturation is called **hematopoiesis.** Numerous growth and differentiation factors that support hematopoiesis have been identified. (See chapter 6.)

Many of the hematopoietic growth factors were identified by culturing bone marrow stem cells *in vitro*. Since each cluster of cells that grew in culture was derived from a single cell, these growth factors were called colony-stimulating factors. There are four main types of colony-stimulating factors: multilineage colony-stimulating factor, also called interleukin–3 (IL–3), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and macrophage colony-stimulating factor (M-CSF). Other cytokines secreted by T-helper lympho-

cytes and macrophages that support hematopoiesis include such factors as interleukins–1, –4, –5, –7, –8, and –9 (IL–1, IL–4, IL–5, IL–7, IL–8 and IL–9). Different growth factors are necessary at different stages of maturation. Multilineage colony-stimulating factor is important early in the differentiation of the myeloid line of blood cells (erythrocytes, monocytes, and granulocytes), whereas G-CSF is important in a later stage of differentiation of the neutrophil line. (See Figure 2.4.)

Granulocytes
Neutrophils, or polymorphonuclear leukocytes, are important early in the inflammatory process and in acute infections. They are the most numerous of all the leukocytes, accounting for approximately 50% to 70% of circulating white blood cells. Neutrophils migrate from the blood stream into the tissue, where they phagocytize pathogens and cellular debris. The ability of these cells to recognize what is antigen (and should be destroyed) is poorly understood but is thought to involve the chemical structure of the antigen. For example, the cell wall of some bacteria contain complex polysaccharides that are not seen in the cell wall of humans. Antibodies, produced by B lymphocytes, may bind to the antigen and mark it for destruction by neutrophils or monocytes. Other circulating proteins known as opsonins may bind to the antigen, also marking it for phagocytosis by neutrophils or monocytes. Basophils contain granules, and have mediators such as histamine, which are important in allergic reactions. In tissues, cells similar to basophils are called mast cells. Eosinophils are phagocytes and play a role in immune responses to parasitic worms. They are present in small numbers in blood: they constitute about 1% to 3% of circulating white blood cells.

Monocytes and the Mononuclear Phagocyte System
Monocytes are large phagocytic cells that comprise approximately 3% to 7% of the circu-

Figure 2.4   Cells of the immune system.

Overview of the Immune System
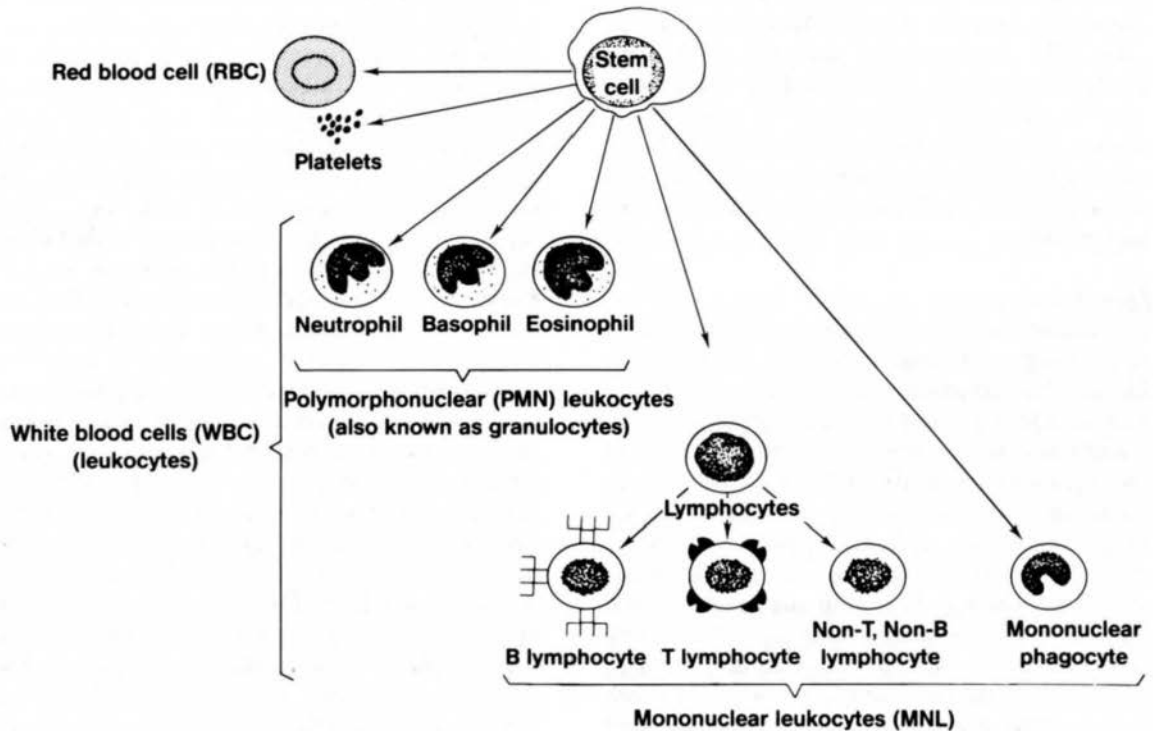
lating white blood cells. They are present late in the inflammatory process and in chronic infections. Tissue monocytes (also called macrophages), when present along with lymphocytes, are often designated as a monocytic infiltrate on **histologic examination.** Macrophages contain many granules and lysosomes and are very effective in removing cellular debris, which is why they are often called the "mop up troops." Many tissues contain resident macrophages, which are called different names in different tissues. For instance, macrophages in the liver are called Kupffer cells; in the skin, Langerhans cells; and in the brain, glial cells. This system of macrophages has

been renamed the mononuclear phagocytic system; it was previously termed the reticuloendothelial system.

Macrophages are not only important in innate responses, but also they play a vital role in adaptive immune responses. Macrophages are often involved in the first stage of processing antigens and presenting them to lymphocyte populations. Foreign microorganisms are phagocytized by the macrophage and then digested in the **phagosomes.** Parts of the digested foreign molecule are further processed inside the cytoplasm; these eventually bind to the macrophage cell-surface proteins and are expressed on the cell surface. The

antigen, in combination with these cell-surface proteins or class II MHC markers, are presented to the lymphocyte. The lymphocyte specific for that antigen then binds to the antigenic determinant and is activated. The cells responsible for processing antigens are also known as **antigen processing cells** or accessory cells. Macrophages can also bind antibodies on their cell surfaces, which will permit them to more readily phagocytize specific antigens. When the antigen and antibody bind, the macrophage is thus activated and becomes more efficient in destroying invading microorganisms.

Lymphocytes

Lymphocytes constitute about 20% to 40% of circulating white blood cells and are responsible for the recognition of antigens and induction of adaptive (specific) immune responses. Lymphocytes also reside in lymphatic tissues throughout the body, which include such structures as the tonsils, adenoids, lymph nodes, thymus, spleen, appendix, Peyer's patches, and bone marrow. The lymphatic tissue underlining the mucous membranes, MALT (mucosa-associated lymphatic tissue), BALT (bronchus-associated lymphatic tissue), and GALT (gut-associated lymphatic tissue), is not **encapsulated**, nor is it as well organized as that of the lymph nodes. In the mucosa, these loose clusters of lymphocytes can interact with antigens at the lumenal surface and prevent antigens from gaining access to the systemic circulation.

Lymphocytes recirculate throughout the body by leaving the vasculature or lymphatic tissue and infiltrating tissues. They then re-enter the circulation via lymphatic vessels and once again take up residence in the lymphatic tissue. Lymphocytes possess adhesion and homing receptors on their surfaces, which permit them to bind to the vascular endothelial cells in general and endothelial cells in specific tissues. The venule endothelial cells possess vascular adhesion molecules which in turn bind with the lymphocyte homing receptors. This recirculation of lymphocytes ensures that antigens come in contact with responsive lymphocytes. It is thought that only 1 in 1,000 to 1 in 100,000 lymphocytes have the ability to respond to a specific antigen.

Although all lymphocytes look similar in a routine blood smear, there are subpopulations. The current method to distinguish cell populations and subpopulations is by staining cell surface molecules with monoclonal antibodies linked to fluorescent dyes. Monoclonal antibodies are highly specific antibodies produced from a single clone of cells. (See Chapter 7.) These surface molecules are called **cluster of differentiation (CD) antigens** (or **CD markers)**. The monoclonal antibodies that are attached to the cell markers and the markers themselves have been assigned CD numbers by an international workshop. Of the three major populations of lymphocytes, T lymphocytes, B lymphocytes, and natural killer (NK) cells, T and B lymphocytes are responsible for specific immune responses and natural killer cells are involved in innate immune reactions to viruses and tumors cells. Although CD5 is a marker for both T and B cells, CD1, CD3, and CD7 are markers only for T cells; CD21, CD22, CD37, and CD40 are a few of the antigens associated with B cells. CD56 is a marker for NK cells. In many cases the function of the cell surface molecule is known. For instance CD21 on B cells is a receptor for a complement molecule and is also the Epstein-Barr virus receptor.

B Lymphocytes In humans, the stem cells for B lymphocytes are located in the bone marrow. In the bird, however, B cells mature in a lymphoid organ called the bursa of Fabricius, which is similar in structure to the human appendix. Since it was in the bird that the function of B cells was first determined, now all lymphocytes that secrete antibodies are called B cells. The molecules on the surface of B cells that are responsible for binding antigen are called membrane-bound immunoglobulins (Ig). On the surface of any one B cell, all

the immunoglobulin receptors are identical in structure and thus bind to only one particular antigen. The most mature form of the B lymphocyte is the **plasma cell**, whose function is to secrete immunoglobulins (i.e., antibodies). All the daughter cells derived from a single B cell will produce the same antibody, therefore the name **monoclonal antibody** is given to the immunoglobulin secreted from a particular clone of B cells. Antibodies or immunoglobulins are found in the plasma, the fluid portion of blood; therefore the historical name associated with the specific immunity produced by B cells is **humoral immunity.** Other names associated with B cell immunity are antibody-mediated immunity and immediate hypersensitivity reactions. (See Figure 2.5).

The B lymphocytes constitute approximately 5% to 15% of circulating lymphocytes; plasma cells, however, do not circulate but remain fixed in the lymphoid tissue. Collections of B cells in lymphoid tissue stain intensely. These collections of B cells are called primary and secondary follicles (or germinal centers). Because B cells predominate, the collections are also called T-independent areas in the lymph nodes. Small amounts of T cells and macrophages are present even in the T-independent areas. Many B cells require the presence of T cells and accessory cells to manufacture antibodies.

T Lymphocytes T lymphocytes are derived from a population of lymphocytes that mature in the thymus. Lymphocyte stem cells migrate from the bone marrow to the thymus in the fetus and in childhood. In the thymus, lymphocyte populations that will react to self molecules (or self antigens) are eliminated and the other T cells differentiate. As the T cells mature, they leave the thymus and migrate to the lymphatic tissue, populating the areas surrounding the germinal centers, paracortical areas. The T lymphocytes constitute about 70% to 80% of circulating lymphocytes. They are responsible for immune reactions such as graft rejection, **graft-versus-host disease**, con-

tact skin sensitivity, and delayed hypersensitivity reactions.

Much of what we know about the T cells was discovered in the nude (hairless) mouse. Because these mice are born without a thymus, they are unable to reject transplanted tissue even if the tissue is from another species. **Nude mice** are often used as incubators of human tumor cell lines because they are unable to reject the foreign tissue. The T lymphocytes are also important regulators of immune responses. As with all physiological responses, regulation of the immune response is necessary to either stimulate or inhibit the reactions.

Like B cells, T cells can recognize and bind to specific antigens. It is the T-cell receptor that binds the antigen. There are two types of T-cell receptors, TCR1 and TCR2; and a particular T cell possesses only one of these types. In the T-cell membrane, the T-cell receptor is linked to a molecule called CD3. The T cell can recognize a foreign antigen only if it is linked to a self molecule (also known as MHC antigens) on the surface of another cell. The T-cell immune responses are particularly effective in eliminating intracellular pathogens, for instance, cells that are infected with a virus and express viral antigens on their cell surfaces. This is in contrast to the immunoglobulins, which can bind to soluble antigens and help eliminate extracellular microorganisms.

The T-lymphocyte population can be further divided into several subpopulations that can be distinguished by the CD4 and CD8 markers. The T4 cells (which express CD4 markers) are called **helper/inducer T-cells.** For the T-helper cells to recognize an antigen, the antigen must be present on another cell that has MHC class II molecules. Thus, T-helper lymphocytes are called class II–restricted cells. These cells secrete cytokines called **lymphokines**, some of which stimulate the growth and differentiation of B lymphocytes and others of which activate macrophages. The T-helper lymphocytes also induce the generation of cytotoxic T lymphocytes. It

Figure 2.5 Differentiation and maturation of T lymphocytes and B lymphocytes. T cells develop in the thymus from cells that originate in the bone marrow. Mature T cells interact with antigen in peripheral tissues and then divide and differentiate into effector T cells. B cells develop in the bone marrow in humans and when mature migrate to peripheral tissues. When stimulated with antigen, they differentiate into antibody-secreting plasma cells and memory cells.
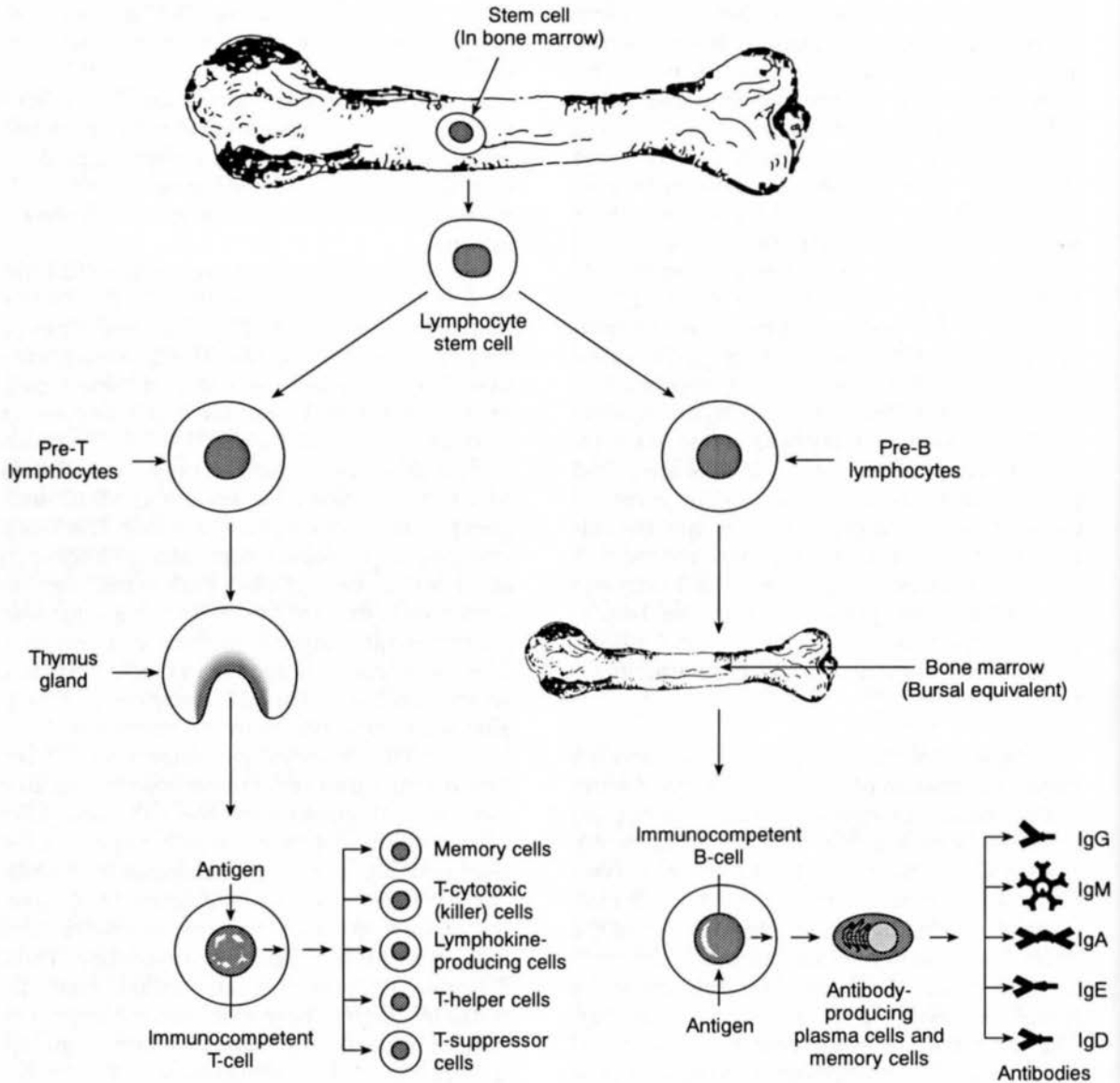
should be noted that the human immunodefi-ciency virus (HIV) appears to bind to the CD4 molecule on T-helper lymphocytes. The selective infection, and ultimate demise of T-helper lymphocytes, explains the amazing severity with which the HIV virus disables the immune system.

The **cytotoxic T cells**, on the other hand, express CD8 markers and can recognize only antigens associated with MHC class I molecules, that is, they are MHC class I–restricted. Cytotoxic T cells can recognize and bind to cells with altered self antigens or viral antigens bound to MHC class I molecules. They release a variety of mediators that cause the altered cell to lyse, or kill, the target cell. The **suppressor T-cells** also have the CD8 markers on their surfaces. They inhibit, or down-regulate, both T and B cell immune reactions and are responsible for tolerance to self. Various monoclonal antibodies are now being developed that would distinguish the cytotoxic T from the T-suppressor subsets and the helper-inducer from the suppressor-inducer subsets. Both helper and suppressor T lymphocytes are necessary for maintaining an appropriate level of immune responsiveness. (See Figure 2.5.)

Other Lymphocytes   Other lymphocytes that are neither B or T lymphocytes include null, natural killer, killer, lymphokine activated killer (LAK), and tumor infiltrating lymphocytes (TIL) cells. **Null cells** are lymphocytes that lack both T and B cell markers; whereas the NK cells and other killer cells carry some T-cell markers or both T-cell and macrophage markers. At one time it was believed that null, NK, and other killer cells were separate and distinct lymphocyte lineages. We now know, however, that as lymphocytes differentiate or become activated, they lose certain markers and gain others. Thus, some subsets may be simply more mature (differentiated) forms of the same line of lymphocytes. Although the exact lineage has not been worked out for all the subsets of lymphocytes, they can be distinguished from each other by their functional properties and cytoplasmic characteristics.

Natural killer cells are large lymphocytes that possess membrane-bound granules that contain **hydrolytic enzymes.** They constitute about 15% of the circulating lymphocytes. These large granular lymphocytes can in culture spontaneously destroy malignant cell lines. Functionally, NK cells can recognize and destroy tumor cells without having prior exposure and are not MHC restricted. Natural killer cells can destroy tumor cells by direct contact and release of perforins, molecules that form doughnut-shaped pores in the target cell membrane. The other process by which killer cells destroy target cells is by **antibody-dependent cell-mediated cytotoxicity (ADCC).** In the process, killer cells bind antibodies to their surfaces. When these antibodies bind to the tumor cell antigens, cytotoxic enzymes are released, resulting in tumor cell destruction. In laboratory studies, NK cells have potent antitumor activity early in tumor development and prevent the metastatic spread of tumors.

Recent biotherapy clinical trials have identified two other subsets of lymphocytes. When lymphocytes are incubated with large concentrations of the lymphokine interleukin–2 (IL–2), they are able to lyse tumor cell lines and fresh tumor cells. These **lymphokine activated killer (LAK) cells** are able to destroy tumor cells more efficiently than normal lymphocytes. **Tumor-infiltrating lymphocytes (TIL)**, are isolated from excised tumors. These lymphocytes, when grown in culture with lower concentrates of IL–2 than LAK cells, appear to be more specific for killing tumor cells derived from the host. Natural killer, LAK, and TIL cells may all be related to each other and derived from the same lymphocyte cell line. Only future studies will determine this. See Table 2.3 for a summary of immune cell functions.

SPECIFIC IMMUNE RESPONSES

The type and the strength of an immune response is dependent on the properties of the

Table 2.3   Immune system cells and their functions

Innate Immunity	
Neutrophils	Phagocytosis; present in acute infections
Basophils	Release of local mediators (histamine); allergic reactions
Eosinophils	Present in allergic reactions
Monocytes	Phagocytosis; present in chronic infections
Natural Killer Cells	Natural immunity to viruses and tumors; expansion of T-cell responses
Adaptive Immunity	
Lymphocytes	
B lymphocytes	Membrane bound Ig binds to antigen
Plasma Cells	Most mature form of B cell, produces Ig
Memory Cell	Responsible for memory effect
T lymphocytes	Membrane bound receptors bind to antigen
T helper	Initiation of immune response, secretion of lymphokines, assist B cell production of antibody
T cytotoxic	Kills cells by lysis
T suppressor	Decreases the immune response of other T- or B-cells
T memory	Responsible for memory effect

antigen, which lymphocyte populations are activated, and the genetic and hormonal characteristics of the individual. The way the antigen is recognized will determine which types of immune responses occur. Each individual produces both B-cell and T-cell responses as well as the innate immune responses to an antigen. In fact, all these responses are interlinked; all are constituents of an integrated defense system. The responses to a microorganism colonizing the host can not always be predicted, however; even the simplest of microbes are very complex and have many molecules that can activate immune cells. Nor is there any guarantee that the immune responses will be beneficial. Indeed, if one type of immune response prevents another more effective response, then the effect is detrimental.
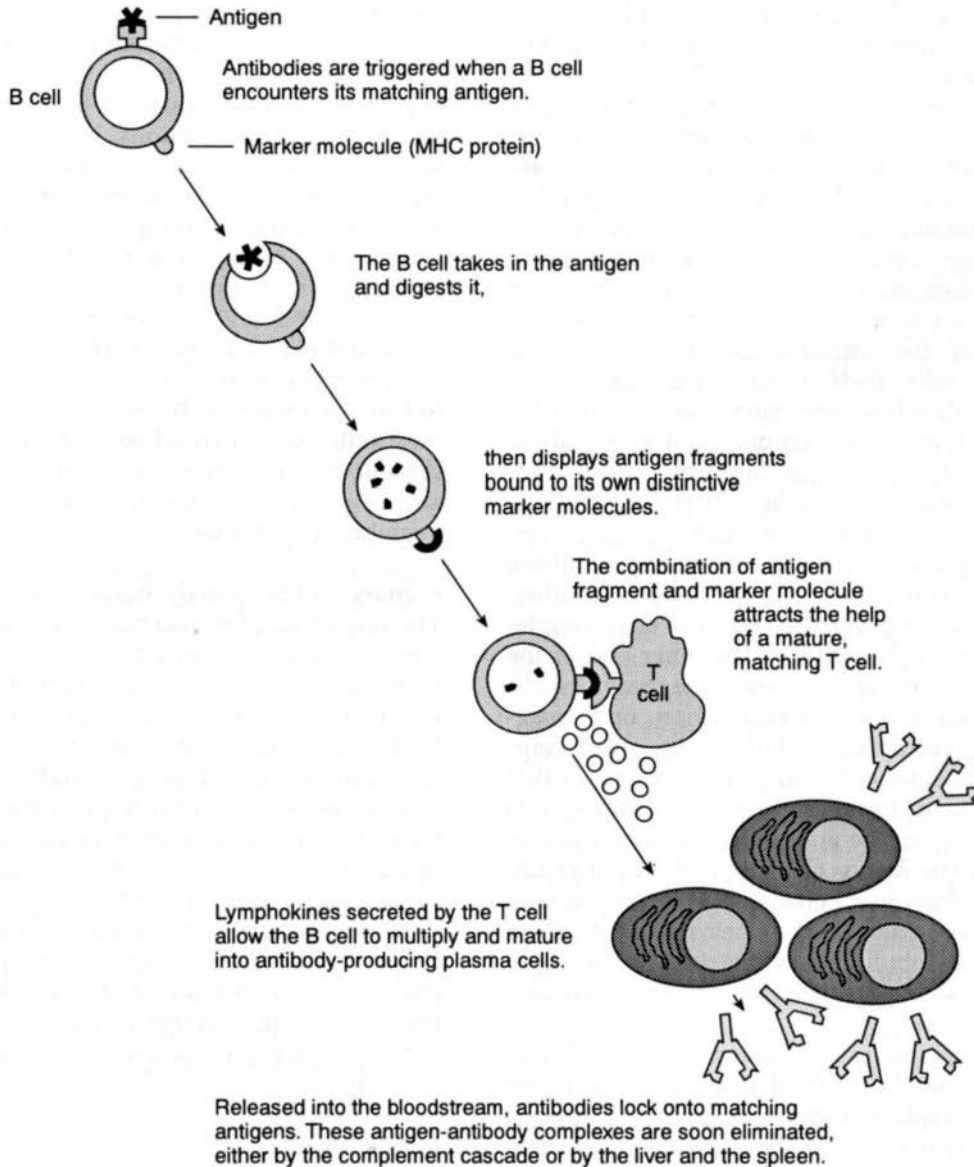
B-Cell Responses

Activation of B Lymphocytes

Resting B lymphocytes are in the G_0 (resting) phase of the cell cycle. When a lymphocyte is activated, it enters the cell cycle and moves through the G_1, S, G_2, and division phases. In the S phase of the cell cycle, DNA is synthesized, whereas in the G (gap) phases, no DNA

is synthesized but RNA, proteins, and other compounds are synthesized. Cytokines act in concert at each of these phases to drive the cell along its cycle, which ends with mitosis or cell division.

The B lymphocytes have on their cell surfaces immunoglobulins that act as receptors for the antigen. The binding of the antigen is a random process. If an antigen binds tightly (fits) with the immunoglobulin receptor, then the activation process starts. The B cell internalizes the antigen and presents it along with a class II MHC molecule to the T cell. The T and B lymphocytes then form a tight bond with each other, a T-B conjugate. The activated T cell secretes cytokines such as IL-2. Thus, the process of B-cell activation requires (1) membrane changes that induce secondary changes within the cytoplasm and nucleus and (2) the presence of cytokines such as IL-1 and IL-4, in the early and IL-2, IL-4, and IL-5 in the later differentiation events. When a B lymphocyte becomes fully differentiated, the antibody is no longer present on the cell surface acting as a receptor but is in the cytoplasm of a plasma cell that is producing large quantities of a specific antibody. (See Figure 2.6.) The process of clonal selection of responding B cells is the basis of the memory response to that antigen.

Figure 2.6 Activation of B cells to produce antibody. Receptors on the surface of B cells bind matching antigen which is then engulfed and processed by the B cell. A piece of the antigen, bound to a class II protein, is then presented on the surface of the B cell. This complex binds to a mature helper T cell, which releases interleukins that transform B cells into antibody-secreting plasma cells. When released into the bloodstream, antibodies lock onto matching antigens and are eliminated by the complement cascade or by the liver and the spleen.

Antigen

B cell

Antibodies are triggered when a B cell encounters its matching antigen.

Marker molecule (MHC protein)

The B cell takes in the antigen and digests it,

then displays antigen fragments bound to its own distinctive marker molecules.

The combination of antigen fragment and marker molecule attracts the help of a mature, matching T cell.

T cell

Lymphokines secreted by the T cell allow the B cell to multiply and mature into antibody-producing plasma cells.

Released into the bloodstream, antibodies lock onto matching antigens. These antigen-antibody complexes are soon eliminated, either by the complement cascade or by the liver and the spleen.

Immunoglobulins

Antibodies are proteins whose function it is to bind the antigen, causing the antigen to lyse, precipitate out, or be more easily phagocytized. The antigen-antibody complex can activate the complement system, or prevent viruses from reacting with receptor sites on cells. When the chemical nature of these proteins are referred to, they are called immunoglobulins. The five main classes of immunoglobulins are IgG, IgM, IgA, IgD, and IgE.

Immunoglobulins are composed of four polypeptide chains. Two of the chains are called the heavy chains and two are called the light chains. This unit of four is also called a monomer. Greek letters have been assigned to the various chains. The light chains are either kappa (κ) or lambda (λ) no matter what the class of the immunoglobulin. The heavy chains take their name from the immunoglobulin class. The heavy chains for IgG, for instance, are called gamma (γ), for IgA, alpha (α), for IgM, mu (μ). Immunoglobulins are bifunctional proteins in that one end of the molecule structure comprises a highly variable region, that is, the amino acid sequence differs greatly from one antibody to another. This is the region which binds to or is complementary to the antigen. The other end of the molecule can bind to membrane receptors and is known as the constant region, or Fc fragment. Macrophages, killer cells, mast cells, and placental cells all have Fc receptors that can bind to immunoglobulins. The fragment that binds to the antigen is composed of one end of the heavy chain and the light chain. The Fc fragment consists of the other end of the heavy chain. Since each monomer of an immunoglobulin has two heavy and two light chains, an immunoglobulin can bind two antigenic sites.

Of all the immunoglobulin classes, IgG is present in the highest concentration in the plasma and is responsible for most specific antibody reactions in the adult. It is the principal antibody secreted in the secondary response and is the antibody passed from the maternal blood to the fetus that is responsible for protection of the fetus and newborn. Two of the four subclasses of IgG are able to activate complement. Also, macrophages and neutrophils have receptors on their cell surfaces that can bind to IgG.

The antibody IgM is formed first. It is the first class of immunoglobulins to be formed in the fetus and the first in primary infections. It is a large molecule, a pentamer, composed of five monomer units, that is very efficient in agglutinating particles such as viruses. Present in mucosal secretions, breast milk, and saliva, IgA helps prevent antigens from gaining access to the systemic circulation. It is the second most abundant immunoglobulin in the serum (10% to 15%). A fourth immunoglobulin, IgE, is present in allergic reactions such as asthma and hay fever and in parasitic infections, and is also known as reaginic antibody. It is present in minute amounts in the plasma, but in the tissues is bound to the surface of mast cells. Not much is known about IgD. It is present in small amounts in the circulation and is involved in the regulation of immunoglobulin synthesis.

Primary and Secondary Responses

The responses of the immune system are different the first time an antigen is encountered from those of second or subsequent times. The first or primary response takes a longer time to develop and is not as specific nor as sustained as the responses to secondary encounters. Whereas in a primary response IgM is formed first and IgG later, in the secondary response IgG titers are much higher than those generated in the primary response, and they are sustained for a longer period of time (Figure 2.7). That is why vaccinations to a particular microorganism are given several times, to generate clones of lymphocytes that can respond more quickly to the presence of an antigen.

Complement

The complement system is a series of about 20 plasma proteins that acts as a bridge between the specific antibody response and the non-

Figure 2.7 Primary and secondary immune responses. Secondary immune responses are distinguished from primary ones by the decrease in the latent period, greater specific and more sustained response.
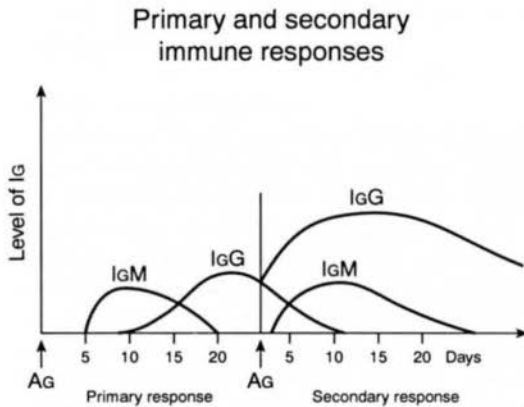
Primary and secondary immune responses

specific inflammatory response. Complement acts to magnify and expand the specific antibody response. The complement proteins circulate as proenzymes or inactive enzymes. When the complement cascade is triggered, often by an antibody-antigen complex, the proteins become active in a series of enzymatic reactions that resemble the blood clotting cascade. The end product of both the classical and the alternative complement pathways is the membrane attack complex. This complex inserts a doughnut-shaped channel into the membrane, which allows lysis of the cell on which the antibody-antigen complex was attached.

When the inactive complement proteins are activated, small polypeptides are released. These small "split products" are nonspecific activators of inflammation and act as chemotactic agents, attracting white blood cells to the area; opsonins, enhancing phagocytosis of the antigen; and anaphylactoid agents, inducing the release of histamine, and increasing

capillary permeability, which causes edema. These functions are vital to the clearance of microorganisms from the host. Individuals who have a congenital deficiency of one of the complement proteins are at higher-than-normal risk of chronic infections or autoimmune diseases.
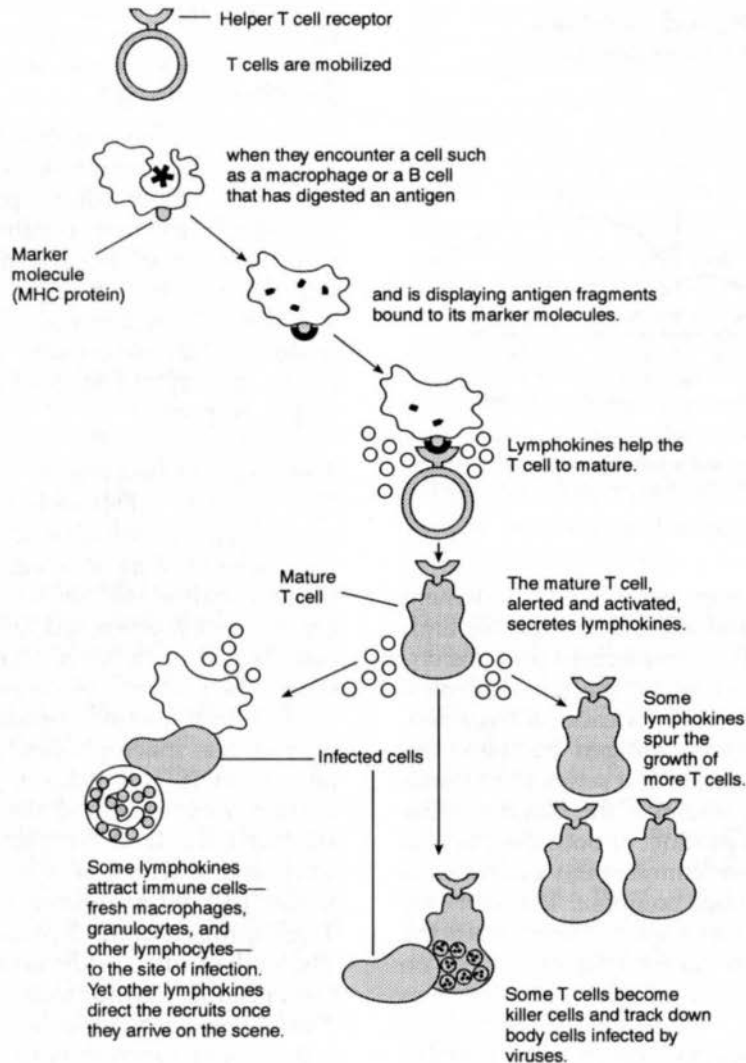
T-Cell Responses

The T cell is responsible for cell-mediated immunity, i.e., immunity carried out by cells. This type of immunity protects the body against intracellular organisms such as viruses and parasites, and malignant cells. T cells are also responsible for immunoregulation through secretion of lymphokines. These responses can be broadly divided into two types, the cytotoxic response and the T-helper response.

T-Helper Cell Responses

The T-helper (CD4) cell is the primary cell responsible for initiation of a specific immune response. Moving a T cell from its resting phase requires the binding of the T-cell receptor with a processed antigen, which is in turn bound to a MHC complex; it also requires the presence of various cytokines such as IL–1. Interleukin–1 produced by accessory cells such as macrophages, causes, along with antigen-MHC stimulation, a change in the plasma membrane and then an activation of IL–2 and the IL–2 receptor genes. This production and secretion of IL–2 and its binding to the IL–2 cell-membrane receptor drives the T cell through to the S phase of the cell cycle. The end result is a differentiated T cell that can produce lymphokines which mediate T-cell immunity, and activate cytotoxic T cells, whose function is to directly lyse cells. The T-helper cell is a major producer of lymphokines.

The T-helper response generates a delayed hypersensitivity reaction, the prototype of which is the positive **PPD** reaction. The results of the PPD test are read after 36 to 72 hours, a delayed response that contrasts with

Figure 2.8 Activation of T cells. After a macrophage internalizes and processes antigen, it presents antigen fragments on its surface in conjunction with class II proteins to the helper T cell. Cytokines secreted by the macrophage help the T cell to mature. The activated T cell then secretes additional lymphokines that attract other immune cells to the area, activate cytotoxic T cells, and cause the growth of more T helper cells.

Helper T cell receptor

T cells are mobilized

when they encounter a cell such as a macrophage or a B cell that has digested an antigen

Marker molecule (MHC protein)

and is displaying antigen fragments bound to its marker molecules.

Lymphokines help the T cell to mature.

Mature T cell

The mature T cell, alerted and activated, secretes lymphokines.

Some lymphokines spur the growth of more T cells.

Infected cells

Some lymphokines attract immune cells— fresh macrophages, granulocytes, and other lymphocytes— to the site of infection. Yet other lymphokines direct the recruits once they arrive on the scene.

Some T cells become killer cells and track down body cells infected by viruses.

the B-cell or immediate hypersensitivity response. A typical immediate hypersensitivity response is a penicillin reaction. The T-cell responses control intracellular pathogens such as *Mycobacteria*, fungi, and viruses, and eliminate altered and malignant cells. Graft rejection and graft-versus-host disease are also T-cell responses.

Cytotoxic T-Cell Responses

The cytotoxic T-cell response requires direct physical contact between the cytotoxic T cell (CD8 lymphocyte) and the target cell (Figure 2.8). Generally, the CD8 cells are MHC class I restricted, meaning that the target cells must have MHC class I antigens expressed on their surfaces. Since almost every cell in the body expresses MHC class I antigens, almost every cell can be killed by cytotoxic T cells.

Before directed cell killing begins, specific T-helper cells are stimulated by the antigen. This expanded pool of T-helper cells then produces IL–2. It is in response to both this IL–2 and the antigen presented with the MHC class I molecule that the resting cytotoxic T cell is stimulated to proliferate and differentiate. The killing of the target cell is the result of a series of steps, starting with the joining or conjugation of the cytotoxic T cell and the target cell. Conjugation is dependent on the binding of the T-cell receptor–CD3 complex with the antigen-MHC class I complex on the target cell. A series of membrane changes occur in the cytotoxic T cell generating a signal in the protoplasm. Orientation of the Golgi complexes and granules in the cytotoxic T cell toward the target cell occurs next, then the contents of the granules are released in the space between the cytotoxic T cell and the target cell. The cytotoxic T cell migrates away and is ready to start the cycle again; shortly after, the target cell lyses. Any one cytotoxic T cell can kill multiple target cells and yet not be harmed itself. The target cell is lysed when pores or channels form in the target cell membrane in a manner reminiscent of complement lysis. However, the proteins required for each type of reaction are different molecules.

TUMOR IMMUNOLOGY

The first experimental system used to study tumor biology was the transplantation of tumors between animals. When this work was started in the late-1800s, inbred strains of animals were unknown, thus, what the scientists were really studying was allogeneic transplantation and responses to antigens. Around the same time, Dr. Coley (see Chapter 1) observed tumor regression in a few patients who had serious infections. He devised a vaccine that would simulate a serious infection and tested it in 1200 patients; 270 of these had complete regression of tumor. Now we know that responses to infections, tumors, and transplantation are all related and that insights into the mechanisms of one are relevant to the other two. The other side of understanding immune responses to infections and transplantation is understanding tolerance to self molecules, an area of study that has helped in discovering the way tumors can evade recognition and destruction by the immune system.

Immune Surveillance and Tumor Antigens

In the 1950s, knowledge of the body's reaction to tumors was synthesized by Mac-Farlane Burnett into a theory of a process termed **immune surveillance**. Expressed in somewhat popular terms, the theory states that (1) all individuals form tumor cells; (2) not everyone develops a malignancy; and (3) it is the immune system that prevents the development of tumors. As stated, the theory can not be directly tested because it is almost impossible to prove the negative, an absence of tumors. However, worded differently, this theory implies that tumors have antigens that under appropriate circumstances can be identified by the immune system. In animal models, the tumor antigens are called tumor transplantation antigens, reflecting the design of the experiments to test these ideas. One way to study tumor rejection and tumor-associated transplantation antigens is to inject

nonviable tumor cells (obtained from another animal of the same inbred line) into an animal as a vaccine. When the animal is later challenged with live tumor cells, it is able to reject the tumor. A control animal without prior exposure to the tumor cell vaccine is unable to reject the live tumor cells and develops a malignancy.

Because transplantation experiments with live tumor cells are not feasible in humans, tumor antigens are termed **tumor-associated antigens** or **oncofetal antigens**. Very few antigens in humans are associated exclusively with malignancy. Most antigens are also present in other pathologic states or in the fetus. Two tumor-associated antigens that are useful clinically are alpha fetoprotein (AFP) and carcinoembryonic antigen (CEA). Carcinoembryonic antigen is expressed in gastrointestinal and breast cancers, AFP in hepatocellular and testicular cancers. Alpha fetoprotein has been used in a few screening programs for the detection of hepatocellular tumors, but these two antigens are more frequently used as indicators of disease progression. Following surgery for the cancers mentioned above, the plasma concentration of the oncofetal antigen usually drops dramatically. If disease recurs, the levels rise once again. A rise in CEA levels might be the first indicator of relapse. However, CEA levels rise in only 60% to 94% of patients, so a negative CEA report does not guarantee an absence of metastases.

Although tumor antigens have been discovered, not all experimentally induced tumors produce antigens recognized by the immune system and some evoke only a "weak" immune response. This is also true of tumors that arise spontaneously, in animals as well as in humans. On the surface of any tumor cell, however, there are many surface marker molecules, such as differentiation antigens, tissue-specific antigens, and receptors, some of which may be exploited for identifying, classifying, and treating the malignancy. Tumor-infiltrating lymphocytes can recognize some tumor-associated antigens.

In the clinic, tumor antigens are detected by monoclonal antibodies to these antigens. As noted above, these monoclonal antibodies can be used in screening and determining tumor progression. They can also be linked to special stains and used in the diagnosis of the tumor type and subtype. For instance, the CD10 antigen, also known as the common acute lymphoblastic leukemia antigen (CALLA), appears in the leukemic cells of approximately 70% of children diagnosed with acute lymphocytic leukemia. This antigen is also seen in Burkitt's and follicular lymphomas. Tumor antigens can also be used to determine the T- or B-cell lineage and the differentiation state of leukemias. Cells positive for CALLA are of the B-cell lineage. Monoclonal antibodies to surface antigens are used to determine the tissue of origin in undifferentiated or poorly differentiated tumors. Monoclonal antibodies can help distinguish tumors of epithelial and connective tissue origins. In clinical trials, monoclonal antibodies are now being linked with radioactive compounds, traditional chemotherapeutic agents, or toxins to determine their utility either in imaging or as therapy. (See Chapter 7.)

Oncogenes, Tumor-Suppressor Genes, and Tumor Antigens

Cancer is a disease of cell growth and division; underlying this abnormal cellular state are genetic changes. The genetic changes that arise in carcinogenesis include point mutation (the change of one nucleotide base in the gene); gene amplification, alterations in the gene control mechanisms; chromosomal deletions and insertions; and chromosomal abnormalities. As the tumor progresses, more and more genetic changes accumulate. One of the main functions of the gene is to code for proteins. Therefore, if carcinogenesis is initiated with a genetic change and that change results in an altered protein, then that protein could be recognized, allowing diagnosis, and become a target for chemotherapy or biological agents.

The genes that have been associated with carcinogenesis are called **oncogenes**. Oncogenes were first recognized in experimental virology, where it was observed that viral genes could transform cells in culture. When these genes were placed into cell cultures, they caused the **phenotype** of the cell to change and take on the appearance of a tumor cell. Since the cells were in a culture, the process was called **transformation** rather than carcinogenesis. It was then discovered that viral oncogenes had a normal cell equivalent, called **proto-oncogenes**. If these genes were first recognized for their normal function, perhaps then they would be called growth, division, and repair genes instead of oncogenes. These genes are responsible for controlling the highly regulated processes of the cell cycle, that is, the sequencing and coordination of the cell through the G_0, G_1, S, G_2, and mitosis phases. The initiating event in cell growth and division is termed a signal. The signal, which might be a growth factor, hormone, cytokine, or antigens, initiates a complex series of actions at the surface membrane and in the cytoplasm and nucleus. Proto-oncogenes are involved in coding for the proteins that act as growth factors, growth factor receptors, and signaling complexes at the membrane; second messengers in the cytoplasm; and regulators of gene expression.

The existence of tumor-suppressor genes or recessive oncogenes was first hypothesized from epidemiologic data. Knudson developed a mathematical model to explain the difference in age of onset of unilateral and bilateral retinoblastoma. He suggested that individuals with familial disease with increased risk for bilateral disease were born with one defective gene of the pair of maternal and paternal genes that control retinoblastoma. (Most genes in the body, except those on the X and Y chromosomes in males, occur as pairs.) The mutation of one allele of the pair of genes occurs earlier in life in patients with familial disease than in those with unilateral disease. Unilateral disease occurs later in life than bilateral disease and requires that mutations occur in both the maternal and paternal genes; since the presence of at least one functioning gene inhibits the growth of the tumor. Great progress has been made in identifying the tumor-suppressor genes in retinoblastoma, Wilms' tumor, von Recklinghausen's neurofibromatosis, familial adenomatous polyposis, and colorectal carcinomas. These genes have a variety of actions, including suppression of cellular transcription factors, DNA replication, and gene transcription.

Clearly, regulation of normal growth and division is complex and under tight control. This complexity in itself may lead to multiple metabolic errors. There are, however, normal cellular mechanisms that limit or correct for these errors. Oncogene products, growth factors, and growth factor receptors are all linked in this complex regulatory system. In malignant diseases, some of the oncogenes are structurally different from their proto-oncogene counterparts. Other oncogenes are expressed in higher amounts in cancer cells than in normal cells, and tumor-suppressor genes may be entirely lacking in the tumor cells. In the future, the protein products of oncogenes might be targets of immunotherapies such as antibodies directed to growth factor receptor produced by an oncogene; the manufacture of suppressor gene products may lead to the development of new biological agents. (See Chapter 16.)

Immune Responses to Tumors

There is some evidence that almost every immune response that has been identified, including cellular and antibody-mediated immunity, is also generated as a response to tumor cells. Probably the most protective responses to tumors are cellular responses, which are similar to those generated against viruses. The important cells in these reactions are cytotoxic T cells, macrophages, NK cells, and antibody-dependent cytotoxic cells. Since the biology of the tumor changes with tumor progression, it may be that the relative importance of one type of immune response differs at the various tumor stages.

Some lines of evidence for an immune response to tumors come from the study of histopathology. To establish a diagnosis of cancer in humans, biopsied tissue is sent to the pathology laboratory, where it is stained and examined. Some tumors have marked infiltrates, often called a mononuclear infiltrate (lymphocytes and macrophages), which suggests that the tumor has stimulated an immune response in the individual. In some instances (medullary carcinoma of the breast, for example) the presence of a mononuclear infiltrate means a more favorable prognosis for the patient. Other clinical signs that have suggested an immune response to tumors is the waxing and waning of tumors. In such cancers as melanomas, the lesions are visible. Some of the lesions will disappear from one area and other tumor deposits will appear simultaneously at distant sites. It has been suggested that the reason for tumor fluctuations is that the immune responses were able to eliminate the tumor from one small area but not to control the progression of the disease in other areas. Spontaneous regression of a diagnosed malignancy is an extremely rare event, but there have been reports of regression in carcinoma of the kidney, neuroblastoma, choriocarcinoma, and melanoma. Again, immune mechanisms appeared to offer the best explanation for the regression. Although clinical evidence suggests the existence of immune responses to tumors, it does not constitute definitive proof. Animal experiments provide further evidence for immune responsiveness to tumors; the development of a cancer-preventing vaccine would be a definitive proof of the theory of immune surveillance.

Cell-Mediated Cytotoxicity

Cytotoxic T cells can recognize and destroy malignant cells by the same mechanisms they use to recognize and destroy cells infected with viruses. The cytotoxic T-cell receptor attaches to the tumor antigen, which is complexed with the class I MHC antigens. The direct contact and binding results in the release of enzymes and lysis of the tumor cell within a few minutes. As with many other specific cytotoxic T-cell reactions, T-helper cell activation is necessary. The T-helper cells are also activated by the presentation of the tumor antigens linked to MHC on macrophages, and activation of T-helper cells can also result in nonspecific cytotoxicity. These cells release lymphokines, which activate macrophages in the area of the tumor. The activated macrophages release lytic enzymes, proteases, and reactive oxygen intermediates which destroy the tumor cells. In another type of cytotoxicity, antibody-dependent cytotoxicity, antibodies bound to a cytotoxic cell, either an NK cell or a macrophage, cross-link the cytotoxic and tumor cells. This binding creates changes in the cell membrane of the macrophage, causing release of lytic granules and death of the tumor cell. Natural killer cells are also responsible for the killing of tumor cells but, unlike the cytotoxic T cells, NK cells do not need prior exposure to generate a cytotoxic response to the tumor cell.

Antitumor Antibodies

In animal experiments, the injection of tumor cells has resulted in the production of antitumor antibodies. The binding of these antibodies to macrophages can lead to tumor cell lysis as described above in antibody-dependent cytotoxicity. Antitumor antibodies could also act as an opsonin. The binding of antibodies to the surface of tumor cells allows phagocytosis by macrophages to occur more readily. Binding of antibodies may also result in the activation of the complement system and the destruction of the tumor cell. Paradoxically, in some animal models it has been shown that the production of antibodies to tumors interfered with cell-mediated antitumor immune responses. Many of the studies in humans of antitumor antibodies have been conducted in patients with melanoma. Very few of these patients, about 6%, generate antitumor antibodies.

Cytokines

Cytokines are low-molecular-weight regulatory proteins produced by various cells. If these proteins are produced by lymphocytes, they are called lymphokines; if by monocytes, they are referred to as **monokines**. Several cytokines are known to have an antitumor effect; they may act alone or in concert with other cytokines. Interferons, tumor necrosis factors, and interleukin–2 are some of the more well-known cytokines with cytotoxic or antiproliferative effects. For instance, interferon gamma activates NK cells, thereby increasing NK cytotoxicity. The interferons are antiproliferative agents and can increase the expression of MHC antigens, which are necessary for T-cell activation. Tumor necrosis factors are secreted by macrophages, NK cells, and T-helper cells. They can directly lyse tumor cells and act in a synergistic fashion with interferon. The cloning of the genes for cytokines and their commercial production has led to an explosion of knowledge about these powerful cell regulators and to their exploitation for therapeutic purposes.

Tumor Escape Mechanisms

Obviously, when a patient is diagnosed with a malignancy, whatever antitumor immune mechanisms were present were not effective. Several different mechanisms are known by which tumors apparently evade the immune system. Antigenic modulation, one of the escape mechanisms, is readily observed in animal models. For instance, an animal that is immunized to a tumor may produce antibodies to the tumor antigen. The antibodies form complexes with the tumor antigen and are taken into the cell by endocytosis or shed to the environment. As long as the antibodies are present, the antigen is lost from the tumor surface and cannot be recognized by a cytotoxic T cell—killing is prevented. Alternatively, shedding of tumor antigens or tumor antigen-antibody complexes may block the activity of the cytotoxic cells.

In humans, as the tumor progresses, it may be that the tumor cells that are most easily recognized by the immune system are killed. Those tumor cells that are left may not express the tumor cell antigen and are able to proliferate without stimulating an immune response. Tumor cells may be able to "sneak through" the immune surveillance network. According to this theory, so few cells and tumor antigens are present early in the growth of a tumor, that the immune system is not stimulated. Later in the natural history of the tumor, there is a sufficient amount of tumor antigen present to stimulate the immune system, but by then the tumor is too large to be controlled by the immune response.

Cell-adhesion molecules are expressed on most somatic cells in the body. These molecules are necessary for the adherence of cytotoxic cells to a target cell before lysis can ensue. During the natural history of tumors, there are multiple genetic changes that are reflected in the phenotype of the cell. One of the changes that occurs in tumor cells is the loss of these cell-adhesion molecules. With the loss of these molecules, cytotoxic cells cannot bind as tightly to the malignant cells and thus the tumor can escape immune surveillance.

Class I MHC molecules on the target cell and the presence of antigen are necessary for cytotoxic T-cell killing. Transformation of cells to a malignant phenotype occurs along with the loss or a decrease in the expression of class I MHC molecules. Theoretically, the loss of these molecules may allow the tumor cell to escape recognition by the cytotoxic cell.

Besides these mechanisms, tumor cells may produce factors that inhibit immune responsiveness. For instance, tumor cells may secrete prostaglandins, which inhibit inflammation and block synthesis of chemotactic factors. This would limit the number of lymphocytes and monocytes in the area of the tumor.

AUTOIMMUNE DISEASES

Autoimmune diseases are another group of diseases in which biological therapies hold great promise. When the immune system reacts against self antigens, the ensuing state is called **autoimmunity.** Approximately 6% of the population suffer a chronic debilitating autoimmune disease. Autoimmune diseases are classified as (1) organ-specific diseases such as Addison's disease, Graves' disease, myasthenia gravis, and poststreptococcal glomerulonephritis or (2) systemic diseases such as ankylosing spondylitis, multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus. The current therapies for autoimmune diseases are mainly those of nonspecific immunosuppression, including pharmacological agents such as steroids and cyclosporine, plasmapheresis, lymph node irradiation, and thymectomy. It has been suggested that more specific biological therapies will become available for these diseases in the future that will have fewer side effects than present therapies and will be directed at the immunological abnormalities.

Autoimmunity is the loss of self tolerance. Normally, lymphocytes that recognize self antigens are eliminated, usually in the fetus, in a process called clonal deletion. Most adults have **clonal anergy,** that is, lymphocytes that can react against self molecules are present in the circulation but do not react. This state of self tolerance must be maintained through regulation of immune responses. As discussed earlier, T-helper cells, T-suppressor cells, the interactions of the MHC antigens and T-cell receptors, and the cytokines are all responsible for regulatory reactions.

All these reactions are currently being studied to determine their role in autoimmune diseases. A great deal of research is now being done to determine the roles of MHC antigens and T-cell receptors in autoimmune diseases. It appears that many autoimmune diseases are associated with MHC class II genes and may result from the inappropriate expression of MHC antigens on cells in the target tissue. The relative risk of developing an autoim-

mune disease is high for individuals who possess certain MHC alleles. Individuals with the HLA-B27 allele, for instance, have a 90% risk of developing ankylosing spondylitis, and those with the HLA-DR3/DQW8 allele have a 100% risk of developing insulin-dependent diabetes. The exact mechanisms of the pathogenesis of these diseases are being explored in animal models, along with specific therapies directed at the T-helper cell receptors and specific T-cell clones. Autoimmune reactions may involve the B-cell system with the production of autoantibodies, the T-cell system with the inappropriate activation of cytotoxic T cells or T-helper cells, or both T-cell and B-cell immune responses.

Current experimental approaches to autoimmune diseases in animal models include the administration of monoclonal antibodies (Kuby, 1992; Wolsy, 1988). In mice with an autoimmune lupus-like syndrome, treatment with monoclonal antibodies to the CD4 molecule (T-helper cell) and to the IL–2 receptor has led to recovery and relief of symptoms. The administration of anti-CD4 antibodies prevented the lymphocytic infiltration of the pancreas and the relief of diabetic symptoms in another experimental animal model (Waldmann, 1988). Monoclonal antibodies to MHC molecules prevented the development of experimentally induced multiple sclerosis. Many strategies other than monoclonal antibody therapies are also being studied for their ability to modify the inflammatory and immune responses responsible for autoimmune diseases, rejections of transplants, and hypersensitivity states.

SUMMARY

The immune system serves as the body's defense system through the functions of protection from pathogenic organisms, surveillance, and homeostasis. Immune responses are divided into two types—innate and adaptive immunity. Innate or nonspecific defenses involve such mechanisms as intact skin and mucous membranes, the acidic environment of

the stomach, the cleansing effect of tears, saliva, exfoliation of the skin, and the inflammatory response. These defenses form the body's first line of defense against pathogens. White blood cells, neutrophils, basophils, and eosinophils are important primarily in nonspecific defenses.

If innate immunity fails, adaptive immunity is enlisted. Specific immune responses recognize foreign antigens while tolerating self molecules. The hallmarks of adaptive immunity are memory and specificity. A additional property of specific immune responses is their ability to react in a more specific and sustained fashion upon the second and subsequent encounters with the antigen. Lymphocytes and macrophages are primarily involved in specific immune reactions. Lymphocytes can be classified as B cells responsible for the production of antibodies (immunoglobulins); T cells are important in cytotoxic and regulatory reactions.

The immunoglobulin receptor on the B cell and the T-cell receptor are responsible for the specific recognition of the antigen. The major histocompatibility genes control interactions among cells of the immune system. These genes code for molecules (MHC antigens) on cell surfaces that are recognized by T lymphocytes which require the antigen to be complexed to self MHC antigens for recognition. Cytokines are powerful mediators released during inflammatory and immune reactions. Although separate, there is significant interaction between innate and adaptive immunity and both are typically required to effect a sufficient response to eliminate pathogens.

The theory of immune surveillance implies that the immune system is capable of recognizing and destroying tumors. When a tumor is diagnosed in a patient, theoretically that tumor has evaded or sneaked through the immune surveillance system. Under appropriate conditions the immune system can recognize tumor associated antigens. T-cell responses to tumor cells resembles immune responses to viruses. B-cell responses to tumors also occur. Both activated macrophages and natural killer cells can lyse tumor cells, natural killer cells without prior exposure.

Rapid advances in technology such as genetic engineering and recombinant DNA technology, coupled with a more complete understanding of the immune response and the molecular basis of oncogenesis have led to the utilization of biological proteins in the treatment of cancer. Numerous cytokines; interferons, interleukins, hematopoietic growth factors, tumor necrosis factor and monoclonal antibodies, are now in clinical investigation or have received regulatory approval. As research continues to progress, new avenues of treatment will become available.

Oncogenes when functioning normally regulate cellular growth, division and differentiation. Suppressor genes or recessive oncogenes inhibit the development of the malignant phenotype in a cell. In the future, protein products of oncogenes or suppressor gene products may be used as biological therapy.

Autoimmune states (glomerulonephritis, systemic lupus, arthritis) are currently being investigated as to the underlying immunologic abnormalities. In the future, biologicals may also be manufactured that will control these illnesses.

References

Boon, T. 1993. Teaching the immune system to fight cancer. *Scientific American* 268 (3): 82–89.

Brostoff, J., Scadding, G., Male, D., et al. (eds.). 1991. *Clinical Immunology*. Philadelphia: J.B. Lippincott.

Burnet, F.M. 1976. *Immunology, aging, and cancer: Medical aspects of mutation and selection*. San Francisco: W.H. Freeman.

Claman, H. 1992. The biology of the immune response. *Journal of the American Medical Association* 268(20): 2790–2796.

Dale, M., and Foreman, J. 1989. *Textbook of Immunopharmacology*. Chicago: Year Book Medical Publishers.

Gallucci, B. 1987. The immune system and cancer. *Oncology Nursing Forum* 14(Suppl 6): 3–12.

Grady, C. 1988. Host defense mechanisms: An overview. *Seminars in Oncology Nursing* 4(2): 86–94.

Griffin, J. (ed). 1986. *Hematology and Immunology: Concepts for Nursing*. Norwalk, CT: Appleton-Century Crofts.

Heberman, R. (ed.). 1993. Miniseries on the Interleukins. *Cancer Investigations* Volume 11.

Hubbard, S., Greene, P., and Knobf, M. (eds). 1993. *Current Issues in Cancer Nursing Practice*. Philadelphia: J.B. Lippincott Co.

Jaffe, H., and Sherwin, S. 1991. Immunomodulators. In Stiles, D., and Terr, A. (eds.). *Basic and Clinical Immunology*. Norwalk, CT: Appleton and Lange, pp. 780–785.

Jaret, P. 1986. Our immune system: the wars within. *National Geographic* 169(6): 702–735.

Knudson, A. 1977. Genetic predisposition to cancer. In Haitt, H., Watson, J., and Winsten, J. (eds.). *Origin of Human Cancer*. Cold Spring Harbor Conferences on Cell Proliferation Volume 4. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, pp. 45–52.

Kuby, J. (ed.). 1992. *Immunology*. New York: W.H. Freeman and Company.

Kuby, J. 1992. Chp 17 Autoimmunity. In Kuby, J. (ed.). *Immunology*. New York: W.H. Freeman and Company, pp. 383–403.

Kunkel, S., and Remick, D. (eds). 1992. *Cytokines in Health and Disease*. New York: Marcel Dekker.

Life, Death and The Immune System. 1993. *Scientific American* 269(3): 52–144.

Mudge-Grout, C. 1992. *Immunologic Disorders*. St. Louis: Mosby Year Book.

Oppenheim, J., and Shevach, E. (eds). 1990. *Immunophysiology: The Role of Cells and Cytokines in Immunity and Inflammation*. New York: Oxford University Press.

Rieger, P., Harle, M., and Rumsey, K. 1992. The Immune System. In Rumsey, K., and Rieger, P. (eds). *Biological Response Modifiers: A Self-Instructional Manual for Health Professionals*. Chicago, IL: Precept Press, Inc., pp. 3–34.

Roitt, I. (ed.). 1991. *Essential Immunology* 7th ed. Boston: Blackwell Scientific Publications.

Roitt, I., Brostoff, J., and Male, D. (eds). 1993. *Immunology* 3rd ed. St. Louis: C.V. Mosby.

Schindler, L. 1992. *The Immune System: How it Works*. U.S. Department of Health and Human Services. Bethesda, MD: National Institutes of Health (NIH publication #92–3229).

Stites, D., and Terr, A. (eds). 1991. *Basic and Clinical Immunology* 7th ed. Norwalk, CT: Appleton and Lange.

Thomson, A. (ed.). 1991. *The Cytokine Handbook*. San Diego: Academic Press, Inc.

Virella, G. (ed.). 1993. *Introduction to Medical Immunology*, 3rd ed. New York: Marcel Dekker.

Waldmann, T. 1986. The structure, function and expression of interleukin-2 receptors on normal and malignant lymphocytes. *Science* 232: 727–732.

Wolsy, D. 1988. Treatment of autoimmune disease with monoclonal antibodies. *Progress in Allergy* 45: 106–120.

Workman, M., and Ellerhorst-Ryan, J. (eds.). *Nursing Care of the Immunocompromised Patient*. Philadelphia, PA: W.B. Sanders.