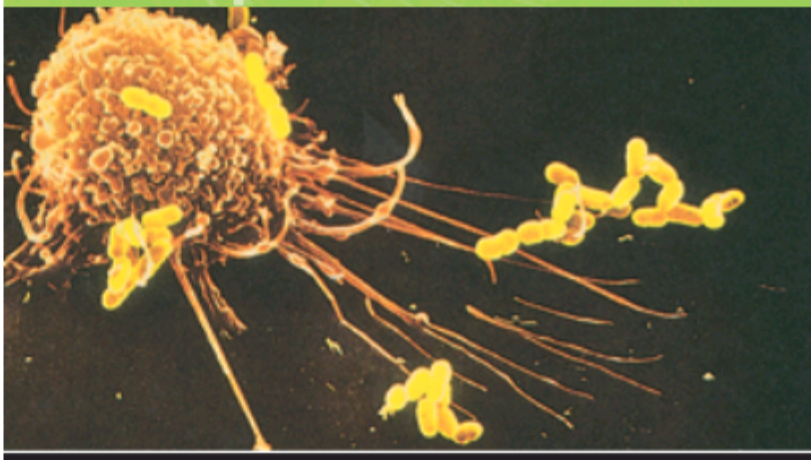
chapter 2

Cells and Organs of the Immune System



Macrophage Interacting with Bacteria

THE IMMUNE SYSTEM CONSISTS OF MANY DIFFERENT organs and tissues that are found throughout the body. These organs can be classified functionally into two main groups. The *primary lymphoid organs* provide appropriate microenvironments for the development and maturation of lymphocytes. The *secondary lymphoid organs* trap antigen from defined tissues or vascular spaces and are sites where mature lymphocytes can interact effectively with that antigen. Blood vessels and lymphatic systems connect these organs, uniting them into a functional whole.

Carried within the blood and lymph and populating the lymphoid organs are various white blood cells, or **leukocytes,** that participate in the immune response. Of these cells, only the lymphocytes possess the attributes of diversity, specificity, memory, and self/nonself recognition, the hallmarks of an adaptive immune response. All the other cells play accessory roles in adaptive immunity, serving to activate lymphocytes, to increase the effectiveness of antigen clearance by phagocytosis, or to secrete various immune-effector molecules. Some leukocytes, especially T lymphocytes, secrete various protein molecules called cytokines. These molecules act as immunoregulatory hormones and play important roles in the regulation of immune responses. This chapter describes the formation of blood cells, the properties of the various immune-system cells, and the functions of the lymphoid organs.

Hematopoiesis

All blood cells arise from a type of cell called the **hematopoietic stem cell (HSC). Stem cells** are cells that can differentiate into other cell types; they are self-renewing—they maintain their population level by cell division. In humans, **hematopoiesis,** the formation and development of red and white blood cells, begins in the embryonic yolk sac during the first weeks of development. Here, yolk-sac stem cells differentiate into primitive erythroid cells that contain embryonic hemoglobin. In the third month of gestation, hematopoietic stem cells migrate from the yolk sac to the fetal liver and then to the spleen; these two organs have major roles in hematopoiesis from the third to the seventh months of gestation. After that, the differentiation of HSCs in the bone marrow becomes the major factor in hematopoiesis, and by birth there is little or no hematopoiesis in the liver and spleen.

It is remarkable that every functionally specialized, mature blood cell is derived from the same type of stem cell. In contrast to a *unipotent* cell, which differentiates into a single cell type, a hematopoietic stem cell is *multipotent,* or *pluripotent,* able to differentiate in various ways and thereby generate erythrocytes, granulocytes, monocytes, mast cells, lymphocytes, and megakaryocytes. These stem cells are few, normally fewer than one HSC per 5×10^4 cells in the bone marrow.

The study of hematopoietic stem cells is difficult both because of their scarcity and because they are hard to grow in vitro. As a result, little is known about how their proliferation and differentiation are regulated. By virtue of their capacity for self-renewal, hematopoietic stem cells are maintained at stable levels throughout adult life; however, when there is an increased demand for hematopoiesis, HSCs display an enormous proliferative capacity. This can be demonstrated in mice whose hematopoietic systems have been completely destroyed by a lethal dose of x-rays (950 rads; one rad represents the absorption by an irradiated target of an amount of radiation corresponding to 100 ergs/gram of target). Such irradiated mice will die within 10 days unless they are infused with normal bone-marrow cells from a syngeneic (genetically identical) mouse. Although a normal mouse has 3×10^8 bone-marrow cells, infusion of only 10^4–10^5 bone-marrow cells (i.e., 0.01%–0.1% of the normal amount) from a donor is sufficient to completely restore the hematopoietic system,

which demonstrates the enormous proliferative and differentiative capacity of the stem cells.

Early in hematopoiesis, a multipotent stem cell differentiates along one of two pathways, giving rise to either a common **lymphoid progenitor cell** or a common **myeloid progenitor cell** (Figure 2-1). The types and amounts of growth factors in the microenvironment of a particular stem cell or progenitor cell control its differentiation. During the development of the lymphoid and myeloid lineages, stem cells differentiate into **progenitor cells,** which have lost the

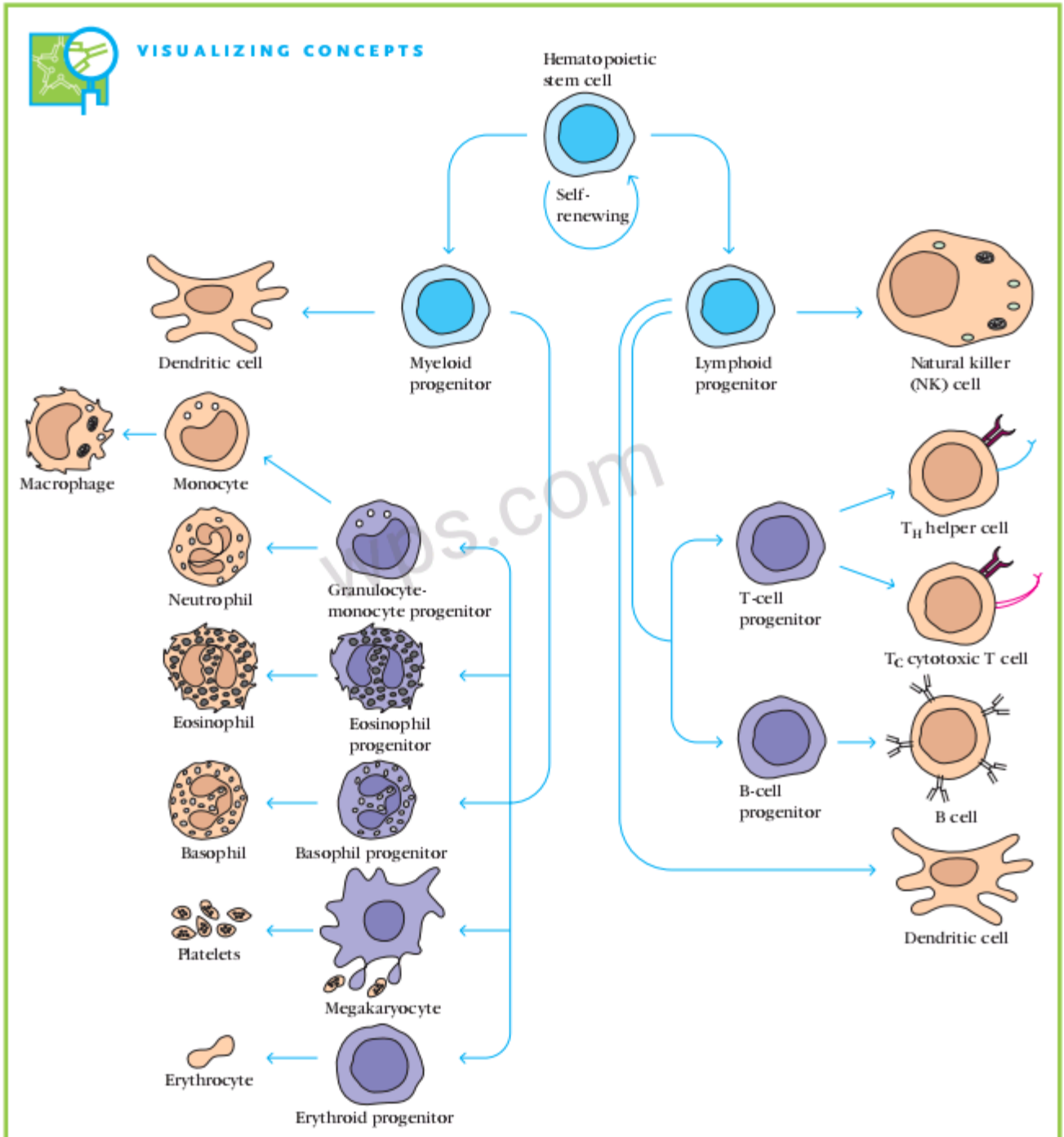


FIGURE 2-1 Hematopoiesis. Self-renewing hematopoietic stem cells give rise to lymphoid and myeloid progenitors. All lymphoid cells descend from lymphoid progenitor cells and all cells of the myeloid lineage arise from myeloid progenitors. Note that some dendritic cells come from lymphoid progenitors, others from myeloid precursors.

capacity for self-renewal and are committed to a particular cell lineage. Common lymphoid progenitor cells give rise to B, T, and NK (natural killer) cells and some dendritic cells. Myeloid stem cells generate progenitors of red blood cells (erythrocytes), many of the various white blood cells (neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells), and platelets. Progenitor commitment depends on the acquisition of responsiveness to particular growth factors and **cytokines.** When the appropriate factors and cytokines are present, progenitor cells proliferate and differentiate into the corresponding cell type, either a mature erythrocyte, a particular type of leukocyte, or a platelet-generating cell (the megakaryocyte). Red and white blood cells pass into bone-marrow channels, from which they enter the circulation.

In bone marrow, hematopoietic cells grow and mature on a meshwork of **stromal cells,** which are nonhematopoietic cells that support the growth and differentiation of hematopoietic cells. Stromal cells include fat cells, endothelial cells, fibroblasts, and macrophages. Stromal cells influence the differentiation of hematopoietic stem cells by providing a **hematopoietic-inducing microenvironment (HIM)** consisting of a cellular matrix and factors that promote growth and differentiation. Many of these hematopoietic growth factors are soluble agents that arrive at their target cells by diffusion, others are membrane-bound molecules on the surface of stromal cells that require cell-to-cell contact between the responding cells and the stromal cells. During infection, hematopoiesis is stimulated by the production of hematopoietic growth factors by activated macrophages and T cells.

Hematopoiesis Can Be Studied In Vitro

Cell-culture systems that can support the growth and differentiation of lymphoid and myeloid stem cells have made it possible to identify many hematopoietic growth factors. In these in vitro systems, bone-marrow stromal cells are cultured to form a layer of cells that adhere to a petri dish; freshly isolated bone-marrow hematopoietic cells placed on this layer will grow, divide, and produce large visible colonies (Figure 2-2). If the cells have been cultured in semisolid agar, their progeny will be immobilized and can be analyzed for cell types. Colonies that contain stem cells can be replated to produce mixed colonies that contain different cell types, including progenitor cells of different cell lineages. In contrast, progenitor cells, while capable of division, cannot be replated and produce lineage-restricted colonies.

Various growth factors are required for the survival, proliferation, differentiation, and maturation of hematopoietic cells in culture. These growth factors, the hematopoietic cytokines, are identified by their ability to stimulate the formation of hematopoietic cell colonies in bone-marrow cultures. Among the cytokines detected in this way was a family of acidic glycoproteins, the **colony-stimulating factors (CSFs),** named for their ability to induce the formation of distinct hematopoietic cell lines. Another important hematopoietic cytokine detected by this method was the glycoprotein **erythropoietin (EPO).** Produced by the kidney, this cytokine induces the terminal development of erythrocytes and regulates the production of red blood cells. Further studies showed that the ability of a given cytokine to signal growth and differentiation is dependent upon the presence of a receptor for that cytokine on the surface of the target cell—commitment of a progenitor cell to a particular differentiation pathway is associated with the expression of membrane receptors that are specific for particular cytokines. Many cytokines and their receptors have since been shown to play essential roles in hematopoiesis. This topic is explored much more fully in the chapter on cytokines (Chapter 11).

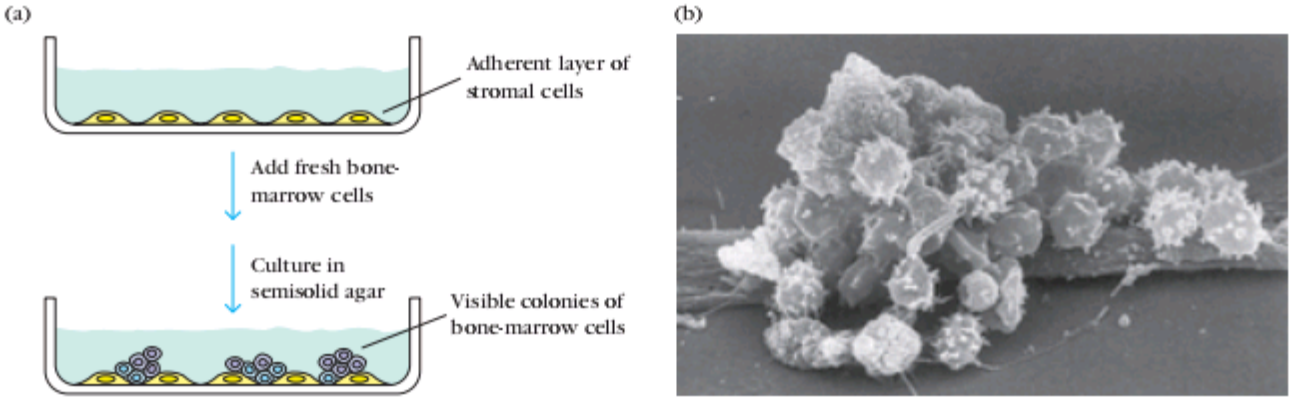


FIGURE 2-2 (a) Experimental scheme for culturing hematopoietic cells. Adherent bone-marrow stromal cells form a matrix on which the hematopoietic cells proliferate. Single cells can be transferred to semisolid agar for colony growth and the colonies analyzed for differentiated cell types. (b) Scanning electron micrograph of cells in long-term culture of human bone marrow. [*Photograph from M. J. Cline and D. W. Golde, 1979,* Nature ***277:180****; reprinted by permission; © 1979 Macmillan Magazines Ltd., micrograph courtesy of S. Quan.*]

Hematopoiesis Is Regulated at the Genetic Level

The development of pluripotent hematopoietic stem cells into different cell types requires the expression of different sets of lineage-determining and lineage-specific genes at appropriate times and in the correct order. The proteins specified by these genes are critical components of regulatory networks that direct the differentiation of the stem cell and its descendants. Much of what we know about the dependence of hematopoiesis on a particular gene comes from studies of mice in which a gene has been inactivated or "knocked out" by targeted disruption, which blocks the production of the protein that it encodes (see Targeted Disruption of Genes, in Chapter 23). If mice fail to produce red cells or particular white blood cells when a gene is knocked out, we conclude that the protein specified by the gene is necessary for development of those cells. Knockout technology is one of the most powerful tools available for determining the roles of particular genes in a broad range of processes and it has made important contributions to the identification of many genes that regulate hematopoiesis.

Although much remains to be done, targeted disruption and other approaches have identified a number of transcription factors (Table 2-1) that play important roles in hematopoiesis. Some of these transcription factors affect many different hematopoietic lineages, and others affect only a single lineage, such as the developmental pathway that leads to lymphocytes. One transcription factor that affects multiple lineages is GATA-2, a member of a family of transcription factors that recognize the tetranucleotide sequence GATA, a nucleotide motif in target genes. A functional ***GATA-2* gene**, which specifies this transcription factor, is essential for the development of the lymphoid, erythroid, and myeloid lineages. As might be expected, animals in which this gene is disrupted die during embryonic development. In contrast to GATA-2, another transcription factor, **Ikaros**, is required only for the development of cells of the lymphoid lineage. Although Ikaros knockout mice do not produce significant numbers of B, T, and NK cells, their production of erythrocytes, granulocytes, and other cells of the myeloid lineage is unimpaired. Ikaros knockout mice survive embryonic development, but they are severely compromised immunologically and die of infections at an early age.

TABLE 2-1 Some transcription factors essential for hematopoietic lineages

Factor	Dependent lineage
GATA-1	Erythroid
GATA-2	Erythroid, myeloid, lymphoid
PU.1	Erythroid (maturational stages), myeloid (later stages), lymphoid
BM11	Myeloid, lymphoid
Ikaros	Lymphoid
Oct-2	B lymphoid (differentiation of B cells into plasma cells)

Hematopoietic Homeostasis Involves Many Factors

Hematopoiesis is a continuous process that generally maintains a steady state in which the production of mature blood cells equals their loss (principally from aging). The average erythrocyte has a life span of 120 days before it is phagocytosed and digested by macrophages in the spleen. The various white blood cells have life spans ranging from a few days, for neutrophils, to as long as 20–30 years for some T lymphocytes. To maintain steady-state levels, the average human being must produce an estimated 3.7×10^{11} white blood cells per day.

Hematopoiesis is regulated by complex mechanisms that affect all of the individual cell types. These regulatory mechanisms ensure steady-state levels of the various blood cells, yet they have enough built-in flexibility so that production of blood cells can rapidly increase tenfold to twentyfold in response to hemorrhage or infection. Steady-state regulation of hematopoiesis is accomplished in various ways, which include:

- Control of the levels and types of cytokines produced by bone-marrow stromal cells
- The production of cytokines with hematopoietic activity by other cell types, such as activated T cells and macrophages
- The regulation of the expression of receptors for hematopoietically active cytokines in stem cells and progenitor cells
- The removal of some cells by the controlled induction of cell death

A failure in one or a combination of these regulatory mechanisms can have serious consequences. For example, abnormalities in the expression of hematopoietic cytokines or their receptors could lead to unregulated cellular proliferation and may contribute to the development of some leukemias. Ultimately, the number of cells in any hematopoietic lineage is set by a balance between the number of cells removed by cell death and the number that arise from division and differentiation. Any one or a combination of regulatory factors can affect rates of cell reproduction and differentiation. These factors can also determine whether a hematopoietic cell is induced to die.

Programmed Cell Death Is an Essential Homeostatic Mechanism

Programmed cell death, an induced and ordered process in which the cell actively participates in bringing about its own demise, is a critical factor in the homeostatic regulation of

many types of cell populations, including those of the hematopoietic system.

Cells undergoing programmed cell death often exhibit distinctive morphologic changes, collectively referred to as **apoptosis** (Figures 2-3, 2-4). These changes include a pronounced decrease in cell volume, modification of the cytoskeleton that results in membrane blebbing, a condensation of the chromatin, and degradation of the DNA into smaller fragments. Following these morphologic changes, an apoptotic cell sheds tiny membrane-bounded apoptotic bodies containing intact organelles. Macrophages quickly phagocytose apoptotic bodies and cells in the advanced stages of apoptosis. This ensures that their intracellular contents, including proteolytic and other lytic enzymes, cationic proteins, and oxidizing molecules are not released into the surrounding tissue. In this way, apoptosis does not induce a local inflammatory response. Apoptosis differs markedly from **necrosis,** the changes associated with cell death arising from injury. In necrosis the injured cell swells and bursts, releasing its contents and possibly triggering a damaging inflammatory response.

Each of the leukocytes produced by hematopoiesis has a characteristic life span and then dies by programmed cell death. In the adult human, for example, there are about 5×10^{10} neutrophils in the circulation. These cells have a life span of only a few days before programmed cell death is initiated. This death, along with constant neutrophil production, maintains a stable number of these cells. If programmed cell death fails to occur, a leukemic state may develop. Programmed cell death also plays a role in maintaining proper numbers of hematopoietic progenitor cells. For example, when colony-stimulating factors are removed, progenitor cells undergo apoptosis. Beyond hematopoiesis, apoptosis is important in such immunological processes as tolerance and the killing of target cells by cytotoxic T cells or natural killer cells. Details of the mechanisms underlying apoptosis are emerging; Chapter 13 describes them in detail.

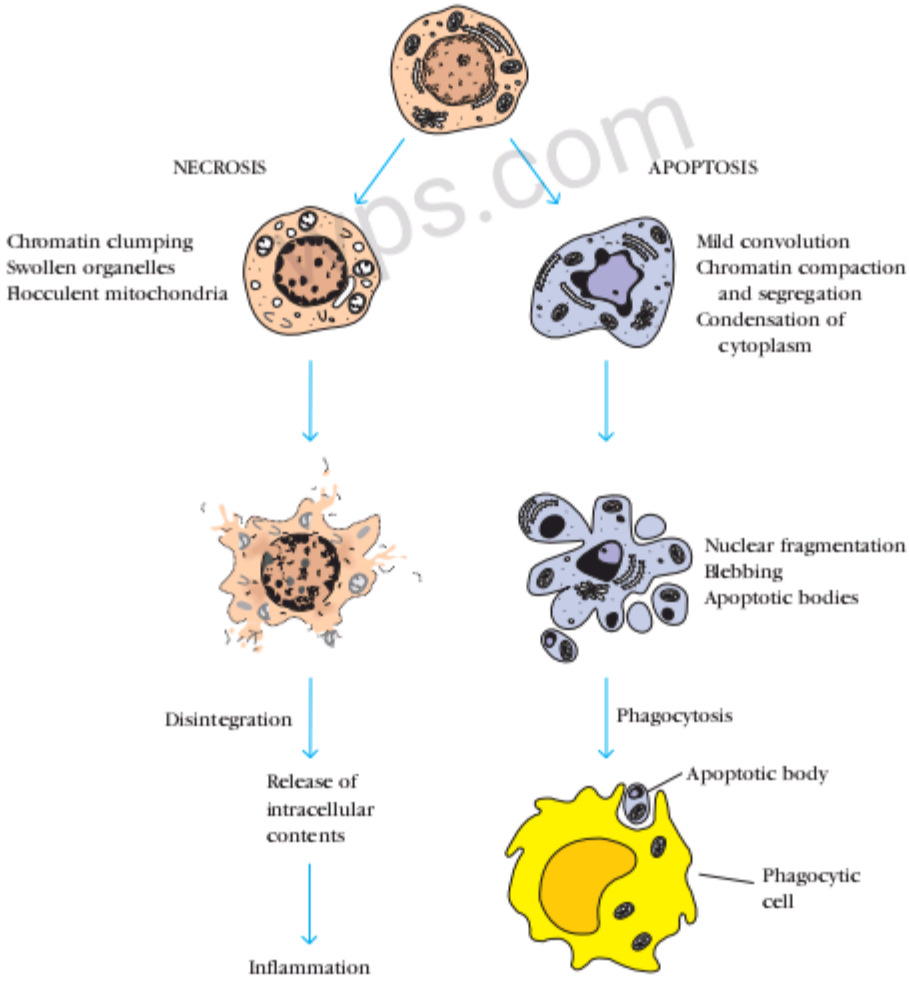


FIGURE 2-3 Comparison of morphologic changes that occur in apoptosis and necrosis. Apoptosis, which results in the programmed cell death of hematopoietic cells, does not induce a local inflammatory response. In contrast, necrosis, the process that leads to death of injured cells, results in release of the cells' contents, which may induce a local inflammatory response.

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Cell Death

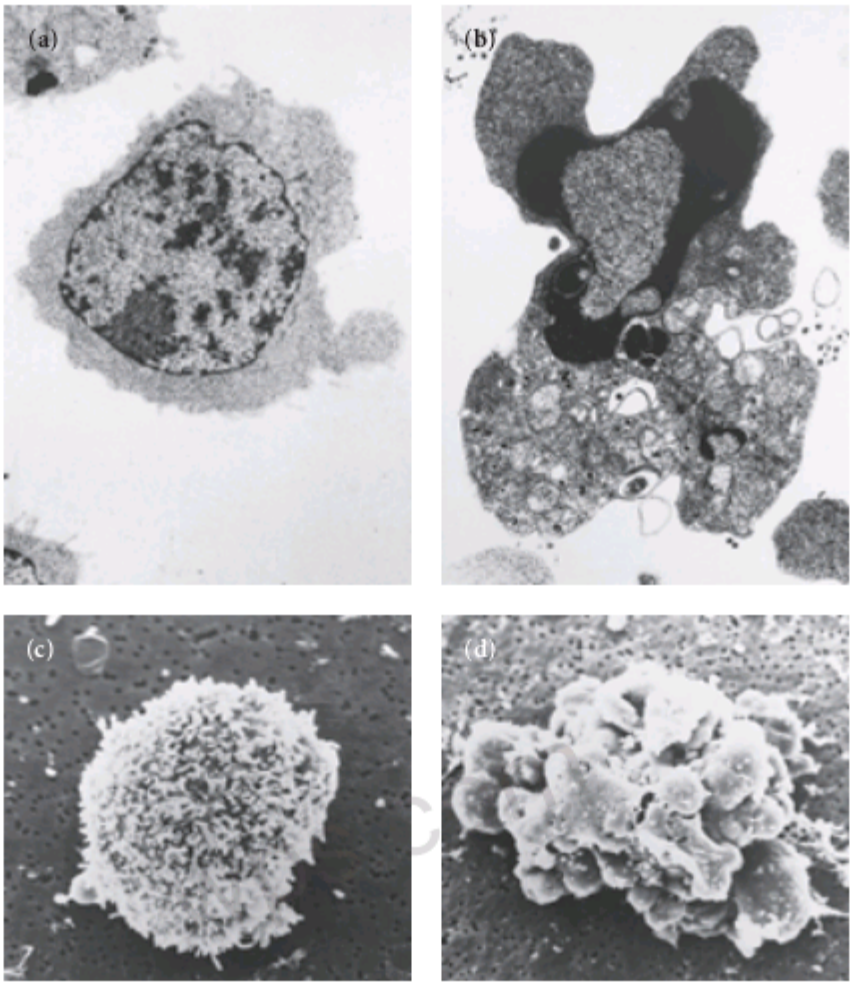

FIGURE 2-4 Apoptosis. Light micrographs of (a) normal thymocytes (developing T cells in the thymus) and (b) apoptotic thymocytes. Scanning electron micrographs of (c) normal and (d) apoptotic thymocytes. [*From B. A. Osborne and S. Smith, 1997,* Journal of NIH Research ***9:35;*** *courtesy B. A. Osborne, University of Massachusetts at Amherst.*]

The expression of several genes accompanies apoptosis in leukocytes and other cell types (Table 2-2). Some of the proteins specified by these genes induce apoptosis, others are critical during apoptosis, and still others inhibit apoptosis. For example, apoptosis can be induced in thymocytes by radiation, but only if the protein p53 is present; many cell deaths are induced by signals from Fas, a molecule present on the surface of many cells; and proteases known as caspases take part in a cascade of reactions that lead to apoptosis. On the other hand, members of the bcl-2 (B-cell lymphoma 2) family of genes, *bcl-2* and *bcl-*X_L encode protein products that inhibit apoptosis. Interestingly, the first member of this gene family, *bcl-2*, was found in studies that were concerned not with cell death but with the uncontrolled proliferation of B cells in a type of cancer known as B-lymphoma. In this case, the *bcl-2* gene was at the breakpoint of a chromosomal translocation in a human B-cell lymphoma. The translocation moved the *bcl-2* gene into the immunoglobulin heavy-chain locus, resulting in transcriptional activation of the *bcl-2* gene and overproduction of the encoded Bcl-2 protein by the lymphoma cells. The resulting high levels of Bcl-2 are thought to help transform lymphoid cells into cancerous lymphoma cells by inhibiting the signals that would normally induce apoptotic cell death.

Bcl-2 levels have been found to play an important role in regulating the normal life span of various hematopoietic cell lineages, including lymphocytes. A normal adult has about 5 L of blood with about 2000 lymphocytes/mm^3 for a total of about 10^{10} lymphocytes. During acute infection, the lymphocyte count increases 4- to 15-fold, giving a total lymphocyte count of $40–50 \times 10^9$. Because the immune system cannot sustain such a massive increase in cell numbers for an extended period, the system needs a means to eliminate unneeded activated lymphocytes once the antigenic threat has passed. Activated lymphocytes have been found to express lower levels of Bcl-2 and therefore are more susceptible to the induction of apoptotic death than are naive lymphocytes or

TABLE 2-2 Genes that regulate apoptosis

Gene	Function	Role in apoptosis
bcl-2	Prevents apoptosis	Inhibits
bax	Opposes *bcl-2*	Promotes
bcl-X$_L$ *(bcl-Long)*	Prevents apoptosis	Inhibits
bcl-X$_S$ *(bcl-Short)*	Opposes *bcl-X*$_L$	Promotes
caspase (several different ones)	Protease	Promotes
fas	Induces apoptosis	Initiates

memory cells. However, if the lymphocytes continue to be activated by antigen, then the signals received during activation block the apoptotic signal. As antigen levels subside, so does activation of the block and the lymphocytes begin to die by apoptosis (Figure 2-5).

Hematopoietic Stem Cells Can Be Enriched

I. L. Weissman and colleagues developed a novel way of enriching the concentration of mouse hematopoietic stem cells, which normally constitute less than 0.05% of all bone-marrow cells in mice. Their approach relied on the use of antibodies specific for molecules known as **differentiation antigens,** which are expressed only by particular cell types. They exposed bone-marrow samples to antibodies that had been labeled with a fluorescent compound and were specific for the differentiation antigens expressed on the surface of mature red and white blood cells (Figure 2-6). The labeled cells were then removed by flow cytometry with a fluorescence-activated cell sorter (see Chapter 6). After each sorting, the remaining cells were assayed to determine the number needed for restoration of hematopoiesis in a lethally x-irradiated mouse. As the pluripotent stem cells were becoming relatively more numerous in the remaining population, fewer and fewer cells were needed to restore hematopoiesis in this system. Because stem cells do not express differentiation antigens

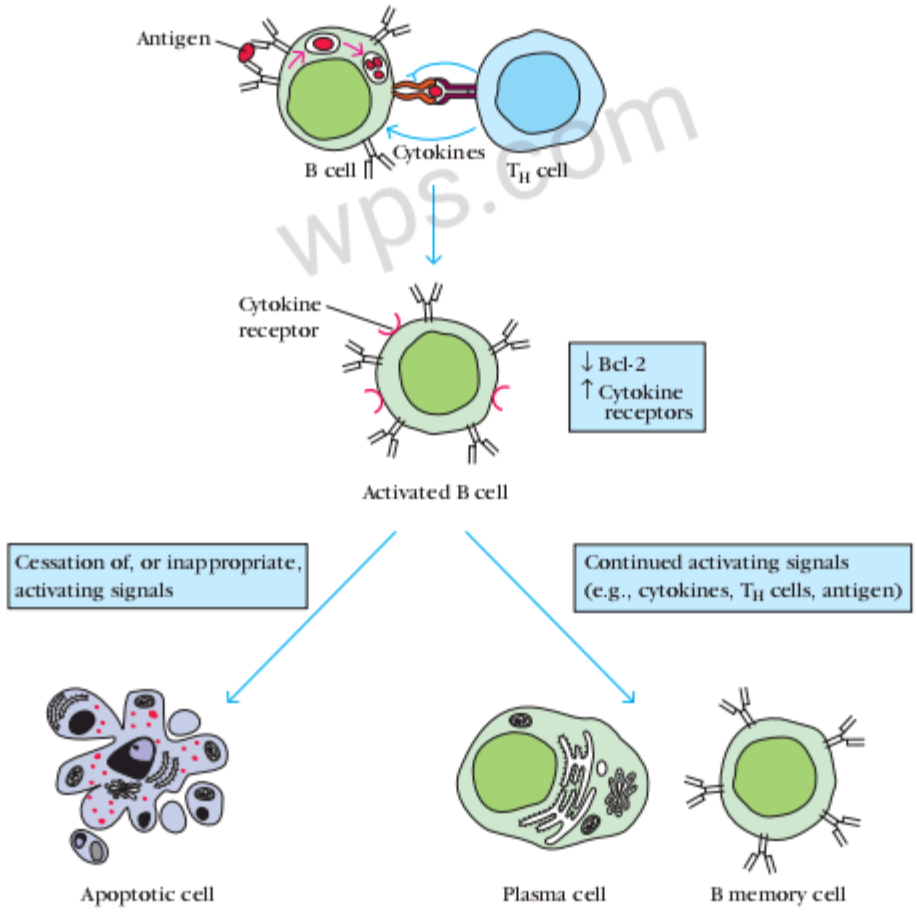

FIGURE 2-5 Regulation of activated B-cell numbers by apoptosis. Activation of B cells induces increased expression of cytokine receptors and decreased expression of Bcl-2. Because Bcl-2 prevents apoptosis, its reduced level in activated B cells is an important factor in making activated B cells more susceptible to programmed cell death than either naive or memory B cells. A reduction in activating signals quickly leads to destruction of excess activated B cells by apoptosis. Similar processes occur in T cells.

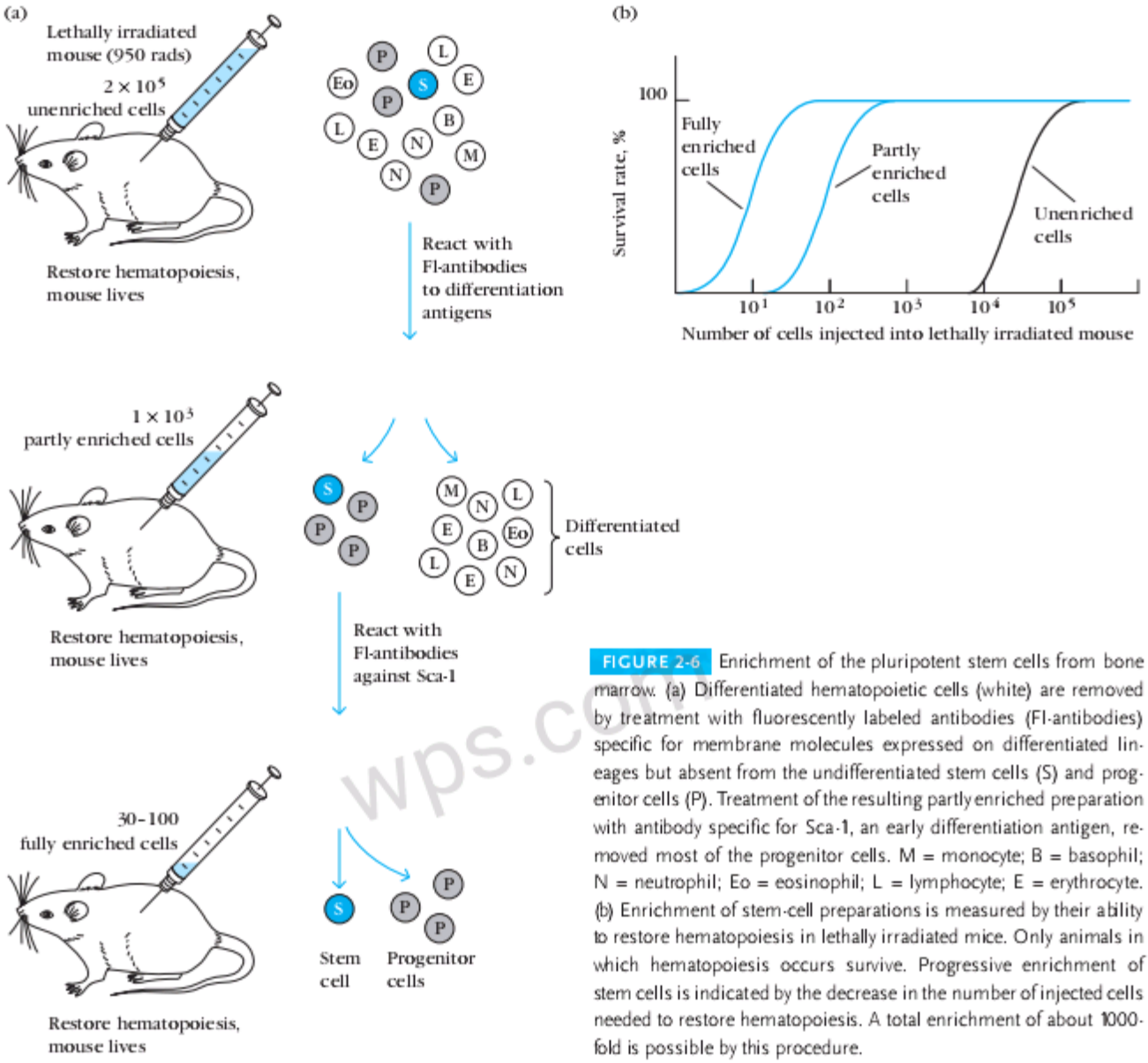

FIGURE 2-6 Enrichment of the pluripotent stem cells from bone marrow. (a) Differentiated hematopoietic cells (white) are removed by treatment with fluorescently labeled antibodies (Fl-antibodies) specific for membrane molecules expressed on differentiated lineages but absent from the undifferentiated stem cells (S) and progenitor cells (P). Treatment of the resulting partly enriched preparation with antibody specific for Sca-1, an early differentiation antigen, removed most of the progenitor cells. M = monocyte; B = basophil; N = neutrophil; Eo = eosinophil; L = lymphocyte; E = erythrocyte. (b) Enrichment of stem-cell preparations is measured by their ability to restore hematopoiesis in lethally irradiated mice. Only animals in which hematopoiesis occurs survive. Progressive enrichment of stem cells is indicated by the decrease in the number of injected cells needed to restore hematopoiesis. A total enrichment of about 1000-fold is possible by this procedure.

known to be on developing and mature hematopoietic cells, by removing those hematopoietic cells that express known differentiation antigens, these investigators were able to obtain a 50- to 200-fold enrichment of pluripotent stem cells. To further enrich the pluripotent stem cells, the remaining cells were incubated with various antibodies raised against cells likely to be in the early stages of hematopoiesis. One of these antibodies recognized a differentiation antigen called stem-cell antigen 1 (Sca-1). Treatment with this antibody aided capture of undifferentiated stem cells and yielded a preparation so enriched in pluripotent stem cells that an aliquot containing only 30–100 cells routinely restored hematopoiesis in a lethally x-irradiated mouse, whereas more than 10^4 nonenriched bone-marrow cells were needed for restoration. Using a variation of this approach, H. Nakauchi and his colleagues have devised procedures that allow them to show that, in 1 out of 5 lethally irradiated mice, a single hematopoietic cell can give rise to both myeloid and lymphoid lineages (Table 2-3).

It has been found that CD34, a marker found on about 1% of hematopoietic cells, while not actually unique to stem cells, is found on a small population of cells that contains stem cells. By exploiting the association of this marker with stem cell populations, it has become possible to routinely enrich preparations of human stem cells. The administration of human-cell populations suitably enriched for $CD34^+$ cells

TABLE 2-3 Reconstitution of hematopoeisis by HSCs

Number of enriched HSCs	Number of mice reconstituted (%)
1	9 of 41 (21.9%)
2	5 of 21 (23.8%)
5	9 of 17 (52.9%)
10	10 of 11 (90.9%)
20	4 of 4 (100%)

SOURCE: Adapted from M. Osawa, et al. 1996. *Science* 273:242.

(the "+" indicates that the factor is present on the cell membrane) can reconstitute a patient's entire hematopoietic system (see Clinical Focus).

A major tool in studies to identify and characterize the human hematopoietic stem cell is the use of **SCID (severe combined immunodeficiency) mice** as in vivo assay systems for the presence and function of HSCs. SCID mice do not have B and T lymphocytes and are unable to mount adaptive immune responses such as those that act in the normal rejection of foreign cells, tissues, and organs. Consequently, these animals do not reject transplanted human cell populations containing HSCs or tissues such as thymus and bone marrow. It is necessary to use immunodeficient mice as surrogate or alternative hosts in human stem-cell research because there is no human equivalent of the irradiated mouse. SCID mice implanted with fragments of human thymus and bone marrow support the differentiation of human hematopoietic stem cells into mature hematopoietic cells. Different subpopulations of $CD34^+$ human bone-marrow cells are injected into these SCID-human mice, and the development of various lineages of human cells in the bone-marrow fragment is subsequently assessed. In the absence of human growth factors, only low numbers of granulocyte-macrophage progenitors develop. However, when appropriate cytokines such as erythropoietin and others are administered along with $CD34^+$ cells, progenitor and mature cells of the myeloid, lymphoid, and erythroid lineages develop. This system has enabled the study of subpopulations of $CD34^+$ cells and the effect of human growth factors on the differentiation of various hematopoietic lineages.

Cells of the Immune System

Lymphocytes are the central cells of the immune system, responsible for adaptive immunity and the immunologic attributes of diversity, specificity, memory, and self/nonself recognition. The other types of white blood cells play important roles, engulfing and destroying microorganisms, presenting antigens, and secreting cytokines.

Lymphoid Cells

Lymphocytes constitute 20%–40% of the body's white blood cells and 99% of the cells in the lymph (Table 2-4). There are approximately 10^{11} (range depending on body size and age: ~10^{10}–10^{12}) lymphocytes in the human body. These lymphocytes continually circulate in the blood and lymph and are capable of migrating into the tissue spaces and lymphoid organs, thereby integrating the immune system to a high degree.

The lymphocytes can be broadly subdivided into three populations—B cells, T cells, and natural killer cells—on the basis of function and cell-membrane components. **Natural killer cells (NK cells)** are large, granular lymphocytes that do not express the set of surface markers typical of B or T cells. Resting B and T lymphocytes are small, motile, nonphagocytic cells, which cannot be distinguished morphologically. B and T lymphocytes that have not interacted with antigen—referred to as **naive**, or unprimed—are resting cells in the G_0 phase of the cell cycle. Known as small lymphocytes, these cells are only about 6 μm in diameter; their cytoplasm forms a barely discernible rim around the nucleus. Small lymphocytes have densely packed chromatin, few mitochondria, and a poorly developed endoplasmic reticulum and Golgi apparatus. The naive lymphocyte is generally thought to have a short life span. Interaction of small lymphocytes with antigen, in the presence of certain cytokines discussed later, induces these cells to enter the cell cycle by progressing from G_0 into G_1 and subsequently into S, G_2, and M (Figure 2-7a). As they progress through the cell cycle, lymphocytes enlarge into 15 μm-diameter blast cells, called **lymphoblasts**; these cells have a higher cytoplasm:nucleus ratio and more organellar complexity than small lymphocytes (Figure 2-7b).

Lymphoblasts proliferate and eventually differentiate into **effector cells** or into **memory cells.** Effector cells function in various ways to eliminate antigen. These cells have short life

TABLE 2-4 Normal adult blood-cell counts

Cell type	Cells/mm³	%
Red blood cells	5.0×10^6	
Platelets	2.5×10^5	
Leukocytes	7.3×10^3	
Neutrophil		50–70
Lymphocyte		20–40
Monocyte		1–6
Eosinophil		1–3
Basophil		<1

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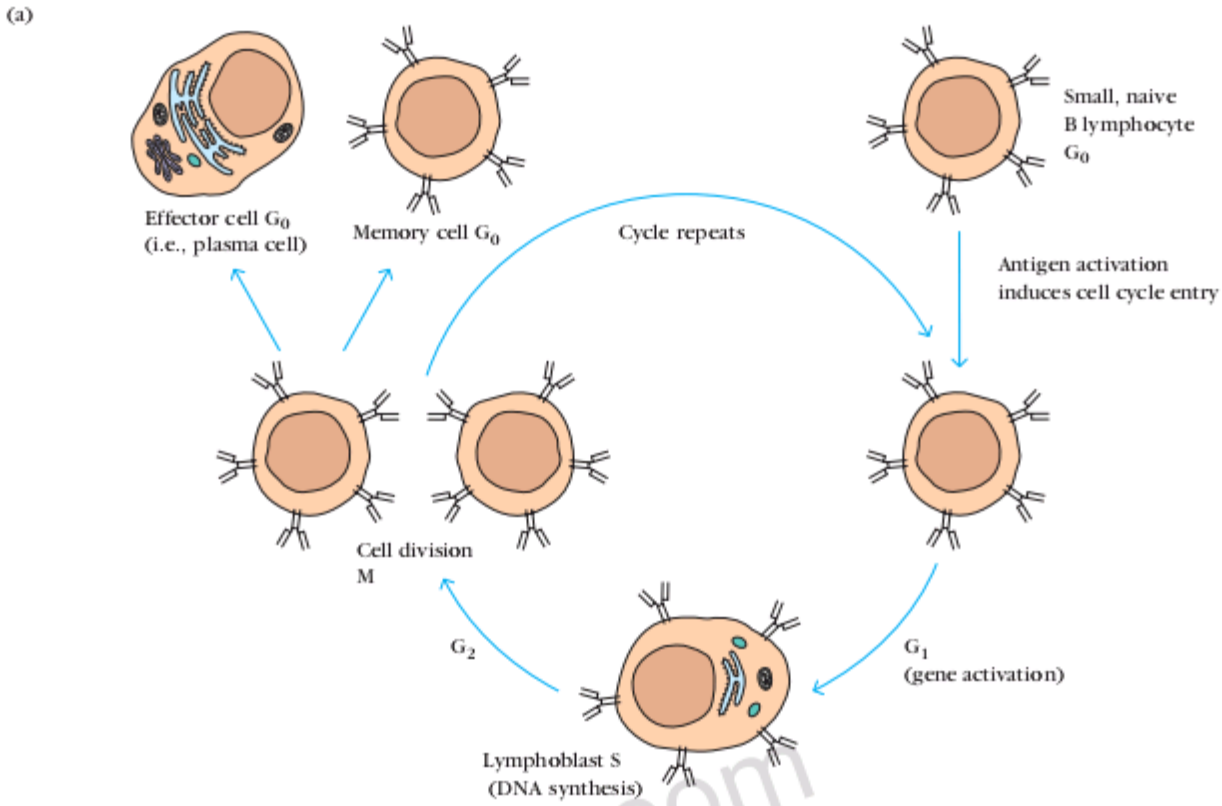
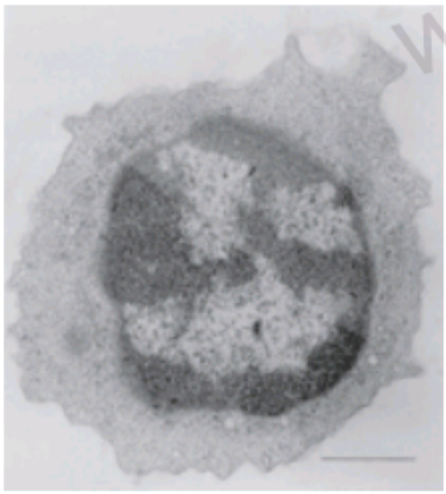
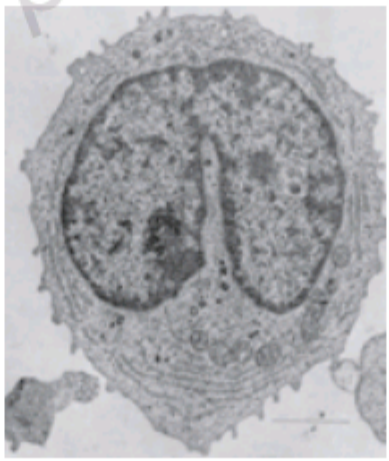


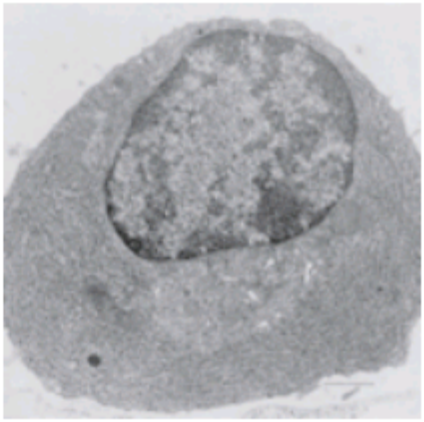
(b)



Small lymphocyte (T or B)
6 µm diameter



Blast cell (T or B)
15 µm diameter



Plasma cell (B)
15 µm diameter

FIGURE 2-7 Fate of antigen-activated small lymphocytes. (a) A small resting (naive or unprimed) lymphocyte resides in the G_0 phase of the cell cycle. At this stage, B and T lymphocytes cannot be distinguished morphologically. After antigen activation, a B or T cell enters the cell cycle and enlarges into a lymphoblast, which undergoes several rounds of cell division and, eventually, generates effector cells and memory cells. Shown here are cells of the B-cell lineage. (b) Electron micrographs of a small lymphocyte (*left*) showing condensed chromatin indicative of a resting cell, an enlarged lymphoblast (*center*) showing decondensed chromatin, and a plasma cell (*right*) showing abundant endoplasmic reticulum arranged in concentric circles and a prominent nucleus that has been pushed to a characteristically eccentric position. The three cells are shown at different magnifications. [*Micrographs courtesy of Dr. J. R. Goodman, Dept. of Pediatrics, University of California at San Francisco.*]

CLINICAL FOCUS

Stem Cells—Clinical Uses and Potential

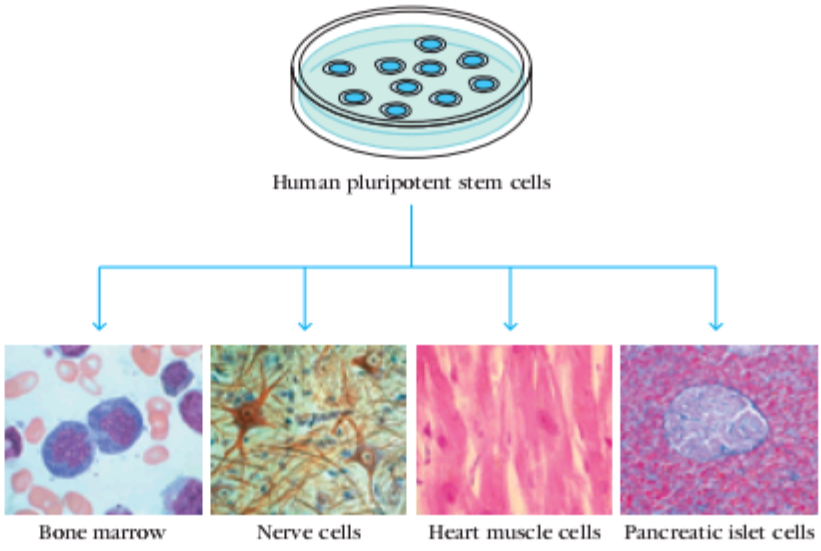
Stem-cell transplantation holds great promise for the regeneration of diseased, damaged, or defective tissue. Hematopoietic stem cells are already used to restore hematopoietic cells, and their use is described in the clinic below. However, rapid advances in stem-cell research have raised the possibility that other stem-cell types, too, may soon be routinely employed for replacement of other cells and tissues. Two properties of stem cells underlie their utility and promise. They have the capacity to give rise to more differentiated cells, and they are self-renewing, because each division of a stem cell creates at least one stem cell. If stem cells are classified according to their descent and developmental potential, four levels of stem cells can be recognized: totipotent, pluripotent, multipotent, and unipotent.

Totipotent cells can give rise to an entire organism. A fertilized egg, the zygote, is a totipotent cell. In humans the initial divisions of the zygote and its descendants produce cells that are also totipotent. In fact, identical twins, each with its own placenta, develop when totipotent cells separate and develop into genetically identical fetuses. Pluripotent stem cells arise from totipotent cells and can give rise to most but not all of the cell types necessary for fetal development. For example, human pluripotent stem cells can give rise to all of the cells of the body but cannot generate a placenta. Further differentiation of pluripotent stem cells leads to the formation of multipotent and unipotent stem cells. Multipotent stem cells can give rise to only a limited number of cell types, and unipotent cells to a single cell type. Pluripotent cells, called embryonic stem cells, or simply ES cells, can be isolated from early embryos, and for many years it has been possible to grow mouse ES cells as cell lines in the laboratory. Strikingly, these ES cells can be induced to generate many different types of cells. Mouse ES cells have been shown to give rise to muscle cells, nerve cells, liver cells, pancreatic cells, and, of course, hematopoietic cells.

Recent advances have made it possible to grow lines of human pluripotent cells. This is a development of considerable importance to the understanding of human development, and it has great therapeutic potential. In vitro studies of the factors that determine or influence the development of human pluripotent stem cells along one developmental path as opposed to another will provide considerable insight into the factors that affect the differentiation of cells into specialized types. There is also great interest in exploring the use of pluripotent stem cells to generate cells and tissues that could be used to replace diseased or damaged ones. Success in this endeavor would be a major advance because transplantation medicine now depends totally upon donated organs and tissues. Unfortunately, the need far exceeds the number of donations and is increasing. Success in deriving practical quantities of cells, tissues, and organs from pluripotent stem cells would provide skin replacement for burn patients, heart muscle cells for those with chronic heart disease, pancreatic islet cells for patients with diabetes, and neurons for use in Parkinson's disease or Alzheimer's disease.

The transplantation of hematopoietic stem cells (HSCs) is an important therapy for patients whose hematopoietic systems must be replaced. It has three major applications:

1. Providing a functional immune system to individuals with a genetically determined immunodeficiency, such as severe



Human pluripotent stem cells can differentiate into a variety of different cell types, some of which are shown here. [*Adapted from* Stem Cells: A Primer, *NIH web site* http://www.nih.gov/news/stemcell/primer.htm. *Micrographs (left to right): Biophoto Associates/Science Source/Photo Researchers; Biophoto Associates/Photo Researchers; AFIP/Science Source/Photo Researchers; Astrid & Hanns-Frieder Michler/Science Photo Library/Photo Researchers.*]

combined immunodeficiency (SCID).

2. Replacing a defective hematopoietic system with a functional one to cure some patients who have a life-threatening nonmalignant genetic disorder in hematopoiesis, such as sickle-cell anemia or thalassemia.
3. Restoring the hematopoietic system of cancer patients after treatment with doses of chemotherapeutic agents and radiation so high that they destroy the system. These high-dose regimens can be much more effective at killing tumor cells than are therapies that use more conventional doses of cytotoxic agents. Stem-cell transplantation makes it possible to recover from such drastic treatment. Also, certain cancers, such as some cases of acute myeloid leukemia, can be cured only by destroying the source of the leukemia cells, the patient's own hematopoietic system.

Restoration of the hematopoietic system by transplanting stem cells is facilitated by several important technical considerations. First, HSCs have extraordinary powers of regeneration. Experiments in mice indicate that only a few—perhaps, on occasion, a single HSC—can completely restore the erythroid population and the immune system. In humans it is necessary to administer as little as 10% of a donor's total volume of bone marrow to provide enough HSCs to completely restore the hematopoietic system. Once injected into a vein, HSCs enter the circulation and find their own way to the bone marrow, where they begin the process of engraftment. There is no need for a surgeon to directly inject the cells into bones. In addition, HSCs can be preserved by freezing. This means that hematopoietic cells can be "banked." After collection, the cells are treated with a cryopreservative, frozen, and then stored for later use. When needed, the frozen preparation is thawed and infused into the patient, where it reconstitutes the hematopoietic system. This cell-freezing technology even makes it possible for individuals to store their own hematopoietic cells for transplantation to themselves at a later time. Currently, this procedure is used to allow cancer patients to donate cells before undergoing chemotherapy and radiation treatments and then to reconstitute their hematopoietic system from their own stem cells. Hematopoietic stem cells are found in cell populations that display distinctive surface antigens. One of these antigens is CD34, which is present on only a small percentage (~1%) of the cells in adult bone marrow. An antibody specific for CD34 is used to select cells displaying this antigen, producing a population enriched in $CD34^+$ stem cells. Various versions of this selection procedure have been used to enrich populations of stem cells from a variety of sources.

Transplantation of stem cell populations may be **autologous** (the recipient is also the donor), **syngeneic** (the donor is genetically identical, i.e., an identical twin of the recipient), or **allogeneic** (the donor and recipient are not genetically identical). In any transplantation procedure, genetic differences between donor and recipient can lead to immune-based rejection reactions. Aside from host rejection of transplanted tissue (host versus graft), lymphocytes in the graft can attack the recipient's tissues, thereby causing **graft-versus-host disease (GVHD)**, a life-threatening affliction. In order to suppress rejection reactions, powerful immunosuppressive drugs must be used. Unfortunately, these drugs have serious side effects, and immunosuppression increases the patient's risk of infection and further growth of tumors. Consequently, HSC transplantation has fewest complications when there is genetic identity between donor and recipient.

At one time, bone-marrow transplantation was the only way to restore the hematopoietic system. However, the essential element of bone-marrow transplantation is really stem-cell transplantation. Fortunately, significant numbers of stem cells can be obtained from other tissues, such as peripheral blood and umbilical-cord blood ("cord blood"). These alternative sources of HSCs are attractive because the donor does not have to undergo anesthesia and the subsequent highly invasive procedure that extracts bone marrow. Many in the transplantation community believe that peripheral blood will replace marrow as the major source of hematopoietic stem cells for many applications. To obtain HSC-enriched preparations from peripheral blood, agents are used to induce increased numbers of circulating HSCs, and then the HSC-containing fraction is separated from the plasma and red blood cells in a process called leukopheresis. If necessary, further purification can be done to remove T cells and to enrich the $CD34^+$ population.

Umbilical cord blood already contains a significant number of hematopoietic stem cells. Furthermore, it is obtained from placental tissue (the "afterbirth") which is normally discarded. Consequently, umbilical cord blood has become an attractive source of cells for HSC transplantation. Although HSCs from cord blood fail to engraft somewhat more often than do cells from peripheral blood, grafts of cord blood cells produce GVHD less frequently than do marrow grafts, probably because cord blood has fewer mature T cells.

Beyond its current applications in cancer treatment, many researchers feel that autologous stem-cell transplantation will be useful for gene therapy, the introduction of a normal gene to correct a disorder caused by a defective gene. Rapid advances in genetic engineering may soon make gene therapy a realistic treatment for genetic disorders of blood cells, and hematopoietic stem cells are attractive vehicles for such an approach. The therapy would entail removing a sample of hematopoietic stem cells from a patient, inserting a functional gene to compensate for the defective one, and then reinjecting the engineered stem cells into the donor. The advantage of using stem cells in gene therapy is that they are self renewing. Consequently, at least in theory, patients would have to receive only a single injection of engineered stem cells. In contrast, gene therapy with engineered mature lymphocytes or other blood cells would require periodic injections because these cells are not capable of self renewal.

spans, generally ranging from a few days to a few weeks. **Plasma cells**—the antibody-secreting effector cells of the B-cell lineage—have a characteristic cytoplasm that contains abundant endoplasmic reticulum (to support their high rate of protein synthesis) arranged in concentric layers and also many Golgi vesicles (see Figure 2-7). The effector cells of the T-cell lineage include the cytokine-secreting T helper cell (T_H cell) and the T cytotoxic lymphocyte (T_C cell). Some of the progeny of B and T lymphoblasts differentiate into memory cells. The persistence of this population of cells is responsible for life-long immunity to many pathogens. Memory cells look like small lymphocytes but can be distinguished from naive cells by the presence or absence of certain cell-membrane molecules.

Different lineages or maturational stages of lymphocytes can be distinguished by their expression of membrane molecules recognized by particular monoclonal antibodies (antibodies that are specific for a single epitope of an antigen; see Chapter 4 for a description of monoclonal antibodies). All of the monoclonal antibodies that react with a particular membrane molecule are grouped together as a **cluster of differentiation (CD)**. Each new monoclonal antibody that recognizes a leukocyte membrane molecule is analyzed for whether it falls within a recognized CD designation; if it does not, it is given a new CD designation reflecting a new membrane molecule. Although the CD nomenclature was originally developed for the membrane molecules of human leukocytes, the homologous membrane molecules of other species, such as mice, are commonly referred to by the same CD designations. Table 2-5 lists some common CD molecules (often referred to as CD markers) found on human lymphocytes. However, this is only a partial listing of the more than 200 CD markers that have been described. A complete list and description of known CD markers is in the appendix at the end of this book.

The general characteristics and functions of B and T lymphocytes were described in Chapter 1 and are reviewed briefly in the next sections. These central cells of the immune system will be examined in more detail in later chapters.

B LYMPHOCYTES

The B lymphocyte derived its letter designation from its site of maturation, in the *b*ursa of Fabricius in birds; the name turned out to be apt, for *b*one marrow is its major site of maturation in a number of mammalian species, including humans and mice. Mature B cells are definitively distinguished from other lymphocytes by their synthesis and display of membrane-bound immunoglobulin (antibody) molecules,

TABLE 2-5 Common CD markers used to distinguish functional lymphocyte subpopulations

			T CELL		
CD designation*	Function	B cell	T_H	T_C	NK cell
CD2	Adhesion molecule; signal transduction	−	+	+	+
CD3	Signal-transduction element of T-cell receptor	−	+	+	−
CD4	Adhesion molecule that binds to class II MHC molecules; signal transduction	−	+ (usually)	− (usually)	−
CD5	Unknown	+	+ (subset)	+	−
CD8	Adhesion molecule that binds to class I MHC molecules; signal transduction	−	− (usually)	+ (usually)	+ (variable)
CD16 (FcγRIII)	Low-affinity receptor for Fc region of IgG	−	−	−	+
CD21 (CR2)	Receptor for complement (C3d) and Epstein-Barr virus	+	−	−	−
CD28	Receptor for co-stimulatory B7 molecule on antigen-presenting cells	−	+	+	−
CD32 (FcγRII)	Receptor for Fc region of IgG	+	−	−	−
CD35 (CR1)	Receptor for complement (C3b)	+	−	−	−
CD40	Signal transduction	+	−	−	−
CD45	Signal transduction	+	+	+	+
CD56	Adhesion molecule	−	−	−	+

*Synonyms are shown in parentheses.

which serve as receptors for antigen. Each of the approximately 1.5×10^5 molecules of antibody on the membrane of a single B cell has an identical binding site for antigen. Among the other molecules expressed on the membrane of mature B cells are the following:

- **B220** (a form of CD45) is frequently used as a marker for B cells and their precursors. However, unlike antibody, it is not expressed uniquely by B-lineage cells.
- **Class II MHC molecules** permit the B cell to function as an antigen-presenting cell (APC).
- **CR1** (CD35) and **CR2** (CD21) are receptors for certain complement products.
- **FcγRII** (CD32) is a receptor for IgG, a type of antibody.
- **B7-1** (CD80) and **B7-2** (CD86) are molecules that interact with CD28 and CTLA-4, important regulatory molecules on the surface of different types of T cells, including T_H cells.
- **CD40** is a molecule that interacts with CD40 ligand on the surface of helper T cells. In most cases this interaction is critical for the survival of antigen-stimulated B cells and for their development into antibody-secreting plasma cells or memory B cells.

Interaction between antigen and the membrane-bound antibody on a mature naive B cell, as well as interactions with T cells and macrophages, selectively induces the activation and differentiation of B-cell clones of corresponding specificity. In this process, the B cell divides repeatedly and differentiates over a 4- to 5-day period, generating a population of plasma cells and memory cells. Plasma cells, which have lower levels of membrane-bound antibody than B cells, synthesize and secrete antibody. All clonal progeny from a given B cell secrete antibody molecules with the same antigen-binding specificity. Plasma cells are terminally differentiated cells, and many die in 1 or 2 weeks.

T LYMPHOCYTES

T lymphocytes derive their name from their site of maturation in the *t*hymus. Like B lymphocytes, these cells have membrane receptors for antigen. Although the antigen-binding T-cell receptor is structurally distinct from immunoglobulin, it does share some common structural features with the immunoglobulin molecule, most notably in the structure of its antigen-binding site. Unlike the membrane-bound antibody on B cells, though, the T-cell receptor (TCR) does not recognize free antigen. Instead the TCR recognizes only antigen that is bound to particular classes of self-molecules. Most T cells recognize antigen only when it is bound to a self-molecule encoded by genes within the major histocompatibility complex (MHC). Thus, as explained in Chapter 1, a fundamental difference between the humoral and cell-mediated branches of the immune system is that the B cell is capable of binding soluble antigen, whereas the T cell is restricted to binding antigen displayed on self-cells. To be recognized by most T cells, this antigen must be displayed together with MHC molecules on the surface of antigen-presenting cells or on virus-infected cells, cancer cells, and grafts. The T-cell system has developed to eliminate these altered self-cells, which pose a threat to the normal functioning of the body.

Like B cells, T cells express distinctive membrane molecules. All T-cell subpopulations express the T-cell receptor, a complex of polypeptides that includes CD3; and most can be distinguished by the presence of one or the other of two membrane molecules, CD4 and CD8. In addition, most mature T cells express the following membrane molecules:

- **CD28,** a receptor for the co-stimulatory B7 family of molecules present on B cells and other antigen-presenting cells
- **CD45,** a signal-transduction molecule

T cells that express the membrane glycoprotein molecule CD4 are restricted to recognizing antigen bound to class II MHC molecules, whereas T cells expressing CD8, a dimeric membrane glycoprotein, are restricted to recognition of antigen bound to class I MHC molecules. Thus the expression of CD4 versus CD8 corresponds to the MHC restriction of the T cell. In general, expression of CD4 and of CD8 also defines two major functional subpopulations of T lymphocytes. $CD4^+$ T cells generally function as T helper (T_H) cells and are class-II restricted; $CD8^+$ T cells generally function as T cytotoxic (T_C) cells and are class-I restricted. Thus the ratio of T_H to T_C cells in a sample can be approximated by assaying the number of $CD4^+$ and $CD8^+$ T cells. This ratio is approximately 2:1 in normal human peripheral blood, but it may be significantly altered by immunodeficiency diseases, autoimmune diseases, and other disorders.

The classification of $CD4^+$ class II–restricted cells as T_H cells and $CD8^+$ dass I–restricted cells as T_C cells is not absolute. Some $CD4^+$ cells can act as killer cells. Also, some T_C cells have been shown to secrete a variety of cytokines and exert an effect on other cells comparable to that exerted by T_H cells. The distinction between T_H and T_C cells, then, is not always clear; there can be ambiguous functional activities. However, because these ambiguities are the exception and not the rule, the generalization of T helper (T_H) cells as being $CD4^+$ and class-II restricted and of T cytotoxic cells (T_C) as being $CD8^+$ and class-I restricted is assumed throughout this text, unless otherwise specified.

T_H cells are activated by recognition of an antigen–class II MHC complex on an antigen-presenting cell. After activation, the T_H cell begins to divide and gives rise to a clone of effector cells, each specific for the same antigen–class II MHC complex. These T_H cells secrete various cytokines, which play a central role in the activation of B cells, T cells, and other cells that participate in the immune response. Changes in the pattern of cytokines produced by T_H cells can change the type of immune response that develops among

other leukocytes. The **T_H1 response** produces a cytokine profile that supports inflammation and activates mainly certain T cells and macrophages, whereas the **T_H2 response** activates mainly B cells and immune responses that depend upon antibodies. T_C cells are activated when they interact with an antigen–class I MHC complex on the surface of an altered self-cell (e.g., a virus-infected cell or a tumor cell) in the presence of appropriate cytokines. This activation, which results in proliferation, causes the T_C cell to differentiate into an effector cell called a **cytotoxic T lymphocyte (CTL)**. In contrast to T_H cells, most CTLs secrete few cytokines. Instead, CTLs acquire the ability to recognize and eliminate altered self-cells.

Another subpopulation of T lymphocytes—called **T suppressor (T_S) cells**—has been postulated. It is clear that some T cells help to suppress the humoral and the cell-mediated branches of the immune system, but the actual isolation and cloning of normal T_S cells is a matter of controversy and dispute among immunologists. For this reason, it is uncertain whether T_S cells do indeed constitute a separate functional subpopulation of T cells. Some immunologists believe that the suppression mediated by T cells observed in some systems is simply the consequence of activities of T_H or T_C subpopulations whose end results are suppressive.

NATURAL KILLER CELLS

The natural killer cell was first described in 1976, when it was shown that the body contains a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumor cells in the absence of any previous immunization with the tumor. NK cells were subsequently shown to play an important role in host defense both against tumor cells and against cells infected with some, though not all, viruses. These cells, which constitute 5%–10% of lymphocytes in human peripheral blood, do not express the membrane molecules and receptors that distinguish T- and B-cell lineages. Although NK cells do not have T-cell receptors or immunoglobulin incorporated in their plasma membranes, they can recognize potential target cells in two different ways. In some cases, an NK cell employs NK cell receptors to distinguish abnormalities, notably a reduction in the display of class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses. Another way in which NK cells recognize potential target cells depends upon the fact that some tumor cells and cells infected by certain viruses display antigens against which the immune system has made an antibody response, so that antitumor or antiviral antibodies are bound to their surfaces. Because NK cells express CD16, a membrane receptor for the carboxyl-terminal end of the IgG molecule, called the Fc region, they can attach to these antibodies and subsequently destroy the targeted cells. This is an example of a process known as **antibody-dependent cell-mediated cytotoxicity (ADCC)**. The exact mechanism of NK-cell cytotoxicity, the focus of much current experimental study, is described further in Chapter 14.

Several observations suggest that NK cells play an important role in host defense against tumors. For example, in humans the **Chediak-Higashi syndrome**—an autosomal recessive disorder—is associated with impairment in neutrophils, macrophages, and NK cells and an increased incidence of lymphomas. Likewise, mice with an autosomal mutation called *beige* lack NK cells; these mutants are more susceptible than normal mice to tumor growth following injection with live tumor cells.

There has been growing recognition of a cell type, the **NK1-T cell**, that has some of the characteristics of both T cells and NK cells. Like T cells, NK1-T cells have T cell receptors (TCRs). Unlike most T cells, the TCRs of NK1-T cells interact with MHC-like molecules called CD1 rather than with class I or class II MHC molecules. Like NK cells, they have variable levels of CD16 and other receptors typical of NK cells, and they can kill cells. A population of triggered NK1-T cells can rapidly secrete large amounts of the cytokines needed to support antibody production by B cells as well as inflammation and the development and expansion of cytotoxic T cells. Some immunologists view this cell type as a kind of rapid response system that has evolved to provide early help while conventional T_H responses are still developing.

Mononuclear Phagocytes

The mononuclear phagocytic system consists of **monocytes** circulating in the blood and **macrophages** in the tissues (Figure 2-8). During hematopoiesis in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes, which leave the bone marrow and enter the blood, where they further differentiate into mature monocytes. Monocytes circulate in the bloodstream for about 8 h, during which they enlarge; they then migrate into the tissues and differentiate into specific tissue macrophages or, as discussed later, into dendritic cells.

Differentiation of a monocyte into a tissue macrophage involves a number of changes: The cell enlarges five- to tenfold; its intracellular organelles increase in both number and complexity; and it acquires increased phagocytic ability, produces higher levels of hydrolytic enzymes, and begins to secrete a variety of soluble factors. Macrophages are dispersed throughout the body. Some take up residence in particular tissues, becoming fixed macrophages, whereas others remain motile and are called free, or wandering, macrophages. Free macrophages travel by amoeboid movement throughout the tissues. Macrophage-like cells serve different functions in different tissues and are named according to their tissue location:

- **Alveolar macrophages** in the lung
- **Histiocytes** in connective tissues
- **Kupffer cells** in the liver
- **Mesangial cells** in the kidney

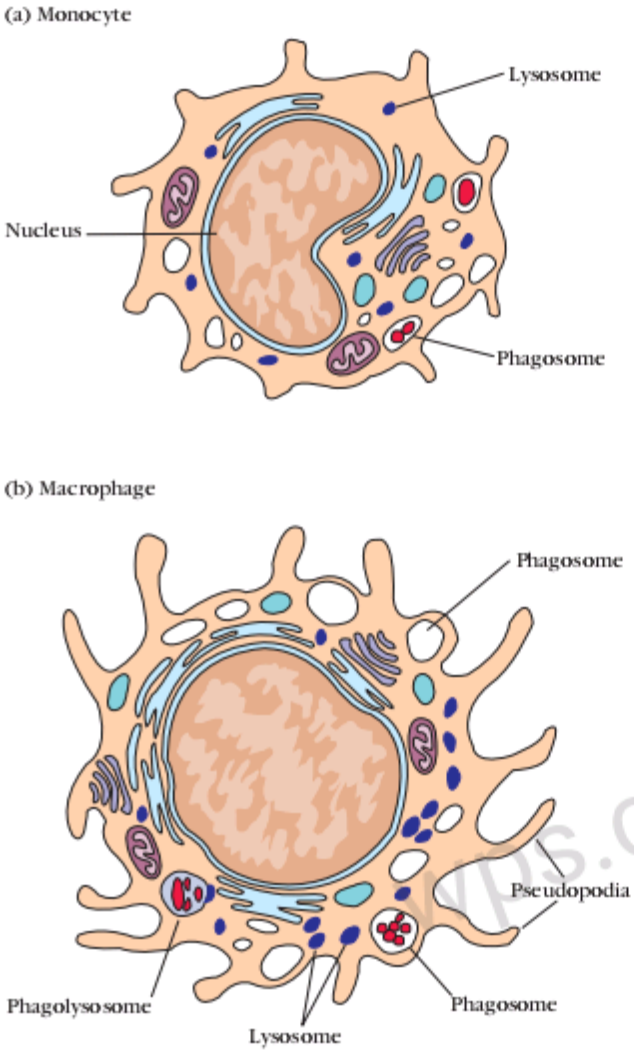


FIGURE 2-8 Typical morphology of a monocyte and a macrophage. Macrophages are five- to tenfold larger than monocytes and contain more organelles, especially lysosomes.

- **Microglial cells** in the brain
- **Osteoclasts** in bone

Although normally in a resting state, macrophages are activated by a variety of stimuli in the course of an immune response. Phagocytosis of particulate antigens serves as an initial activating stimulus. However, macrophage activity can be further enhanced by cytokines secreted by activated T_H cells, by mediators of the inflammatory response, and by components of bacterial cell walls. One of the most potent activators of macrophages is interferon gamma (IFN-γ) secreted by activated T_H cells.

Activated macrophages are more effective than resting ones in eliminating potential pathogens, because they exhibit greater phagocytic activity, an increased ability to kill ingested microbes, increased secretion of inflammatory mediators, and an increased ability to activate T cells. In addition, activated macrophages, but not resting ones, secrete various cytotoxic proteins that help them eliminate a broad range of pathogens, including virus-infected cells, tumor cells, and intracellular bacteria. Activated macrophages also express higher levels of class II MHC molecules, allowing them to function more effectively as antigen-presenting cells. Thus, macrophages and T_H cells facilitate each other's activation during the immune response.

PHAGOCYTOSIS

Macrophages are capable of ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles, and endogenous matter, such as injured or dead host cells, cellular debris, and activated clotting factors. In the first step in phagocytosis, macrophages are attracted by and move toward a variety of substances generated in an immune response; this process is called **chemotaxis.** The next step in phagocytosis is adherence of the antigen to the macrophage cell membrane. Complex antigens, such as whole bacterial cells or viral particles, tend to adhere well and are readily phagocytosed; isolated proteins and encapsulated bacteria tend to adhere poorly and are less readily phagocytosed. Adherence induces membrane protrusions, called **pseudopodia,** to extend around the attached material (Figure 2-9a). Fusion of the pseudopodia encloses the material within a membrane-bounded structure called a **phagosome,** which then enters the endocytic processing pathway (Figure 2-9b). In this pathway, a phagosome moves toward the cell interior, where it fuses with a **lysosome** to form a **phagolysosome.** Lysosomes contain lysozyme and a variety of other hydrolytic enzymes that digest the ingested material. The digested contents of the phagolysosome are then eliminated in a process called **exocytosis** (see Figure 2-9b).

The macrophage membrane has receptors for certain classes of antibody. If an antigen (e.g., a bacterium) is coated with the appropriate antibody, the complex of antigen and antibody binds to antibody receptors on the macrophage membrane more readily than antigen alone and phagocytosis is enhanced. In one study, for example, the rate of phagocytosis of an antigen was 4000-fold higher in the presence of specific antibody to the antigen than in its absence. Thus, antibody functions as an **opsonin,** a molecule that binds to both antigen and macrophage and enhances phagocytosis. The process by which particulate antigens are rendered more susceptible to phagocytosis is called **opsonization.**

ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES

A number of antimicrobial and cytotoxic substances produced by activated macrophages can destroy phagocytosed microorganisms (Table 2-6). Many of the mediators of cytotoxicity listed in Table 2-6 are reactive forms of oxygen.

OXYGEN-DEPENDENT KILLING MECHANISMS Activated phagocytes produce a number of **reactive oxygen intermediates (ROIs)** and **reactive nitrogen intermediates** that have

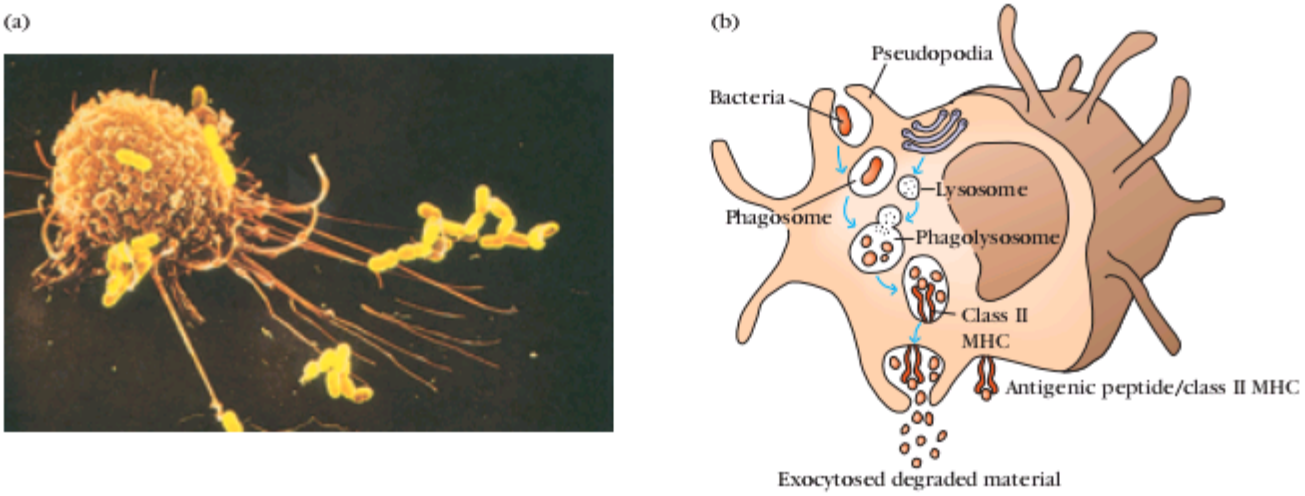


FIGURE 2-9 Macrophages can ingest and degrade particulate antigens, including bacteria. (a) Scanning electron micrograph of a macrophage. Note the long pseudopodia extending toward and making contact with bacterial cells, an early step in phagocytosis. (b) Phagocytosis and processing of exogenous antigen by macrophages. Most of the products resulting from digestion of ingested material are exocytosed, but some peptide products may interact with class II MHC molecules, forming complexes that move to the cell surface, where they are presented to T_H cells. [*Photograph by L. Nilsson, © Boehringer Ingelheim International GmbH.*]

potent antimicrobial activity. During phagocytosis, a metabolic process known as the **respiratory burst** occurs in activated macrophages. This process results in the activation of a membrane-bound oxidase that catalyzes the reduction of oxygen to superoxide anion, a reactive oxygen intermediate that is extremely toxic to ingested microorganisms. The superoxide anion also generates other powerful oxidizing agents, including hydroxyl radicals and hydrogen peroxide. As the lysosome fuses with the phagosome, the activity of myeloperoxidase produces hypochlorite from hydrogen peroxide and chloride ions. Hypochlorite, the active agent of household bleach, is toxic to ingested microbes. When macrophages are activated with bacterial cell-wall components such as lipopolysaccharide (LPS) or, in the case of mycobacteria, muramyl dipeptide (MDP), together with a T-cell–derived cytokine (IFN-γ), they begin to express high levels of **nitric oxide synthetase (NOS)**, an enzyme that oxidizes L-arginine to yield L-citrulline and **nitric oxide (NO)**, a gas:

$$\text{L-arginine} + O_2 + \text{NADPH} \longrightarrow \text{NO} + \text{L-citrulline} + \text{NADP}$$

Nitric oxide has potent antimicrobial activity; it also can combine with the superoxide anion to yield even more potent antimicrobial substances. Recent evidence suggests that much of the antimicrobial activity of macrophages against bacteria, fungi, parasitic worms, and protozoa is due to nitric oxide and substances derived from it.

TABLE 2-6 Mediators of antimicrobial and cytotoxic activity of macrophages and neutrophils

Oxygen-dependent killing	Oxygen-independent killing
Reactive oxygen intermediates	Defensins
$O^{\cdot}_2{}^-$ (superoxide anion)	Tumor necrosis factor α
$OH^{\cdot}$ (hydroxyl radicals)	(macrophage only)
H_2O_2 (hydrogen peroxide)	Lysozyme
ClO^- (hypochlorite anion)	Hydrolytic enzymes
Reactive nitrogen intermediates	
NO (nitric oxide)	
NO_2 (nitrogen dioxide)	
HNO_2 (nitrous acid)	
Others	
NH_2CL (monochloramine)	

OXYGEN-INDEPENDENT KILLING MECHANISMS Activated macrophages also synthesize **lysozyme** and various hydrolytic enzymes whose degradative activities do not require oxygen. In addition, activated macrophages produce a group of antimicrobial and cytotoxic peptides, commonly known as **defensins.** These molecules are cysteine-rich cationic peptides containing 29–35 amino-acid residues. Each peptide, which contains six invariant cysteines, forms a circular molecule that is stabilized by intramolecular disulfide bonds. These circularized defensin peptides have been shown to form ion-permeable channels in bacterial cell membranes. Defensins can kill a variety of bacteria, including *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli,*

Pseudomonas aeruginosa, and *Haemophilus influenzae*. Activated macrophages also secrete **tumor necrosis factor α (TNF-α)**, a cytokine that has a variety of effects and is cytotoxic for some tumor cells.

ANTIGEN PROCESSING AND PRESENTATION

Although most of the antigen ingested by macrophages is degraded and eliminated, experiments with radiolabeled antigens have demonstrated the presence of antigen peptides on the macrophage membrane. As depicted in Figure 2-9b, phagocytosed antigen is digested within the endocytic processing pathway into peptides that associate with class II MHC molecules; these peptide–class II MHC complexes then move to the macrophage membrane. Activation of macrophages induces increased expression of both class II MHC molecules and the co-stimulatory B7 family of membrane molecules, thereby rendering the macrophages more effective in activating T_H cells. This processing and presentation of antigen, examined in detail in Chapter 7, are critical to T_H-cell activation, a central event in the development of both humoral and cell-mediated immune responses.

SECRETION OF FACTORS

A number of important proteins central to development of immune responses are secreted by activated macrophages (Table 2-7). These include a collection of cytokines, such as **interleukin 1 (IL-1)**, TNF-α and **interleukin 6 (IL-6)**, that promote inflammatory responses. Typically, each of these agents has a variety of effects. For example, IL-1 activates lymphocytes; and IL-1, IL-6, and TNF-α promote fever by affecting the thermoregulatory center in the hypothalamus.

TABLE 2-7 Some factors secreted by activated macrophages

Factor	Function
Interleukin 1 (IL-1)	Promotes inflammatory responses and fever
Interleukin 6 (IL-6), TNF-α	Promote innate immunity and elimination of pathogens
Complement proteins	Promote inflammatory response and elimination of pathogens
Hydrolytic enzymes	Promote inflammatory response
Interferon alpha (IFN-α)	Activates cellular genes, resulting in the production of proteins that confer an antiviral state on the cell
Tumor necrosis factor (TNF-α)	Kills tumor cells
GM-CSF, G-CSF, M-CSF	Promote inducible hematopoiesis

Activated macrophages secrete a variety of factors involved in the development of an inflammatory response. The **complement proteins** are a group of proteins that assist in eliminating foreign pathogens and in promoting the ensuing inflammatory reaction. The major site of synthesis of complement proteins is the liver, although these proteins are also produced in macrophages. The hydrolytic enzymes contained within the lysosomes of macrophages also can be secreted when the cells are activated. The buildup of these enzymes within the tissues contributes to the inflammatory response and can, in some cases, contribute to extensive tissue damage. Activated macrophages also secrete soluble factors, such as TNF-α, that can kill a variety of cells. The secretion of these cytotoxic factors has been shown to contribute to tumor destruction by macrophages. Finally, as mentioned earlier, activated macrophages secrete a number of cytokines that stimulate inducible hematopoiesis.

Granulocytic Cells

The **granulocytes** are classified as neutrophils, eosinophils, or basophils on the basis of cellular morphology and cytoplasmic staining characteristics (Figure 2-10). The **neutrophil** has a multilobed nucleus and a granulated cytoplasm that stains with both acid and basic dyes; it is often called a polymorphonuclear leukocyte (PMN) for its multilobed nucleus. The **eosinophil** has a bilobed nucleus and a granulated cytoplasm that stains with the acid dye eosin red (hence its name). The **basophil** has a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye methylene blue. Both neutrophils and eosinophils are phagocytic, whereas basophils are not. Neutrophils, which constitute 50%–70% of the circulating white blood cells, are much more numerous than eosinophils (1%–3%) or basophils (<1%).

NEUTROPHILS

Neutrophils are produced by hematopoiesis in the bone marrow. They are released into the peripheral blood and circulate for 7–10 h before migrating into the tissues, where they have a life span of only a few days. In response to many types of infections, the bone marrow releases more than the usual number of neutrophils and these cells generally are the first to arrive at a site of inflammation. The resulting transient increase in the number of circulating neutrophils, called **leukocytosis**, is used medically as an indication of infection.

Movement of circulating neutrophils into tissues, called **extravasation**, takes several steps: the cell first adheres to the vascular endothelium, then penetrates the gap between adjacent endothelial cells lining the vessel wall, and finally penetrates the vascular basement membrane, moving out into the tissue spaces. (This process is described in detail in Chapter 15.) A number of substances generated in an inflammatory reaction serve as **chemotactic factors** that promote accumulation of neutrophils at an inflammatory site. Among these chemotactic factors are some of the complement

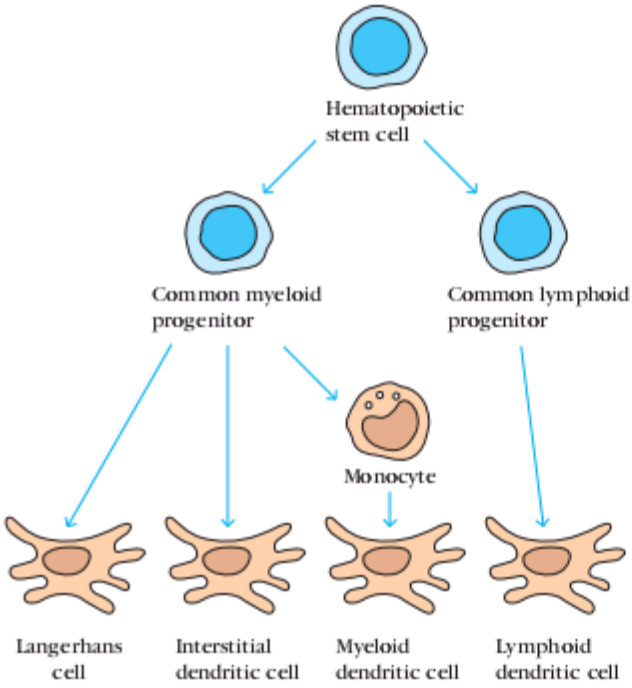


FIGURE 2-11 Dendritic cells arise from both the myeloid and lymphoid lineages. The myeloid pathway that gives rise to the monocyte/macrophage cell type also gives rise to dendritic cells. Some dendritic cells also arise from the lymphoid lineage. These considerations do not apply to follicular dendritic cells, which are not derived from bone marrow.

they all constitutively express high levels of both class II MHC molecules and members of the co-stimulatory B7 family. For this reason, they are more potent antigen-presenting cells than macrophages and B cells, both of which need to be activated before they can function as antigen-presenting cells (APCs). Immature or precursor forms of each of these types of dendritic cells acquire antigen by phagocytosis or endocytosis; the antigen is processed, and mature dendritic cells present it to T_H cells. Following microbial invasion or during inflammation, mature and immature forms of Langerhans cells and interstitial dendritic cells migrate into draining lymph nodes, where they make the critical presentation of antigen to T_H cells that is required for the initiation of responses by those key cells.

Another type of dendritic cell, the **follicular dendritic cell** (Figure 2-12), does not arise in bone marrow and has a different function from the antigen-presenting dendritic cells described above. Follicular dendritic cells do not express class II MHC molecules and therefore do not function as antigen-presenting cells for T_H-cell activation. These dendritic cells were named for their exclusive location in organized structures of the lymph node called lymph follicles, which are rich in B cells. Although they do not express class II molecules, follicular dendritic cells express high levels of membrane receptors for antibody, which allows the binding of antigen-antibody complexes. The interaction of B cells with this bound antigen can have important effects on B cell responses.

Organs of the Immune System

A number of morphologically and functionally diverse organs and tissues have various functions in the development of immune responses. These can be distinguished by function as the **primary** and **secondary lymphoid organs** (Figure 2-13). The thymus and bone marrow are the primary (or central) lymphoid organs, where maturation of lymphocytes takes place. The lymph nodes, spleen, and various mucosal-associated lymphoid tissues (MALT) such as gut-associated lymphoid tissue (GALT) are the secondary (or peripheral) lymphoid organs, which trap antigen and provide sites for mature lymphocytes to interact with that antigen. In addition, **tertiary lymphoid tissues,** which normally contain fewer lymphoid cells than secondary lymphoid organs, can import lymphoid cells during an inflammatory response. Most prominent of these are cutaneous-associated lymphoid tissues. Once mature lymphocytes have been generated in the primary lymphoid organs, they circulate in the blood and **lymphatic system,** a network of vessels that collect fluid that has escaped into the tissues from capillaries of the circulatory system and ultimately return it to the blood.

Primary Lymphoid Organs

Immature lymphocytes generated in hematopoiesis mature and become committed to a particular antigenic specificity within the primary lymphoid organs. Only after a lympho-

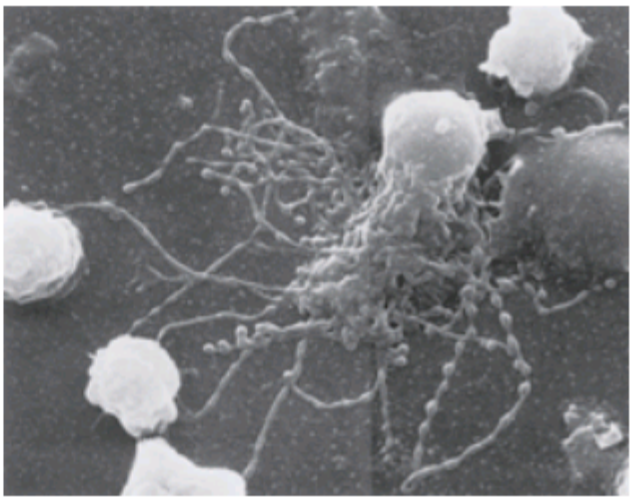


FIGURE 2-12 Scanning electron micrograph of follicular dendritic cells showing long, beaded dendrites. The beads are coated with antigen-antibody complexes. The dendrites emanate from the cell body. [*From A. K. Szakal et al., 1985,* J. Immunol. ***134:1353;*** *© 1996 by American Association of Immunologists, reprinted with permission.*]

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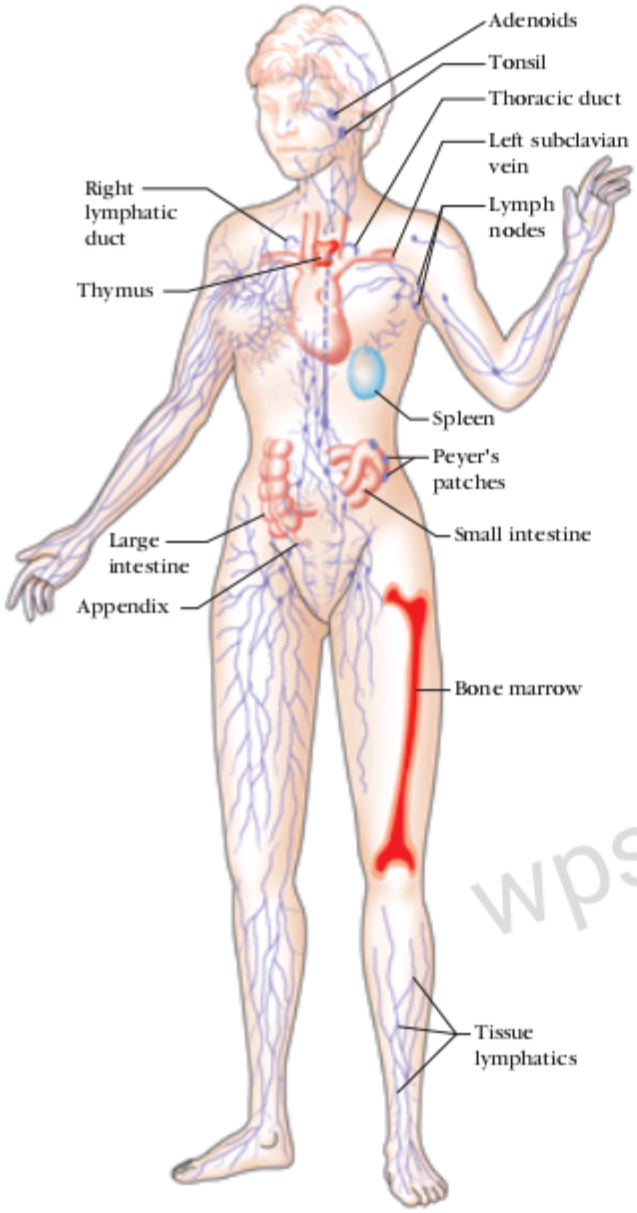

FIGURE 2-13 The human lymphoid system. The primary organs (bone marrow and thymus) are shown in red; secondary organs and tissues, in blue. These structurally and functionally diverse lymphoid organs and tissues are interconnected by the blood vessels (not shown) and lymphatic vessels (purple) through which lymphocytes circulate. Only one bone is shown, but all major bones contain marrow and thus are part of the lymphoid system. [*Adapted from H. Lodish et al., 1995,* Molecular Cell Biology, *3rd ed., Scientific American Books.*]

cyte has matured within a primary lymphoid organ is the cell **immunocompetent** (capable of mounting an immune response). T cells arise in the **thymus,** and in many mammals—humans and mice for example—B cells originate in **bone marrow.**

THYMUS

The thymus is the site of T-cell development and maturation. It is a flat, bilobed organ situated above the heart. Each lobe is surrounded by a capsule and is divided into lobules, which are separated from each other by strands of connective tissue called trabeculae. Each lobule is organized into two compartments: the outer compartment, or *cortex,* is densely packed with immature T cells, called thymocytes, whereas the inner compartment, or *medulla,* is sparsely populated with thymocytes.

Both the cortex and medulla of the thymus are crisscrossed by a three-dimensional stromal-cell network composed of epithelial cells, dendritic cells, and macrophages, which make up the framework of the organ and contribute to the growth and maturation of thymocytes. Many of these stromal cells interact physically with the developing thymocytes (Figure 2-14). Some thymic epithelial cells in the outer cortex, called **nurse cells,** have long membrane extensions that surround as many as 50 thymocytes, forming large multicellular complexes. Other cortical epithelial cells have long interconnecting cytoplasmic extensions that form a network and have been shown to interact with numerous thymocytes as they traverse the cortex.

The function of the thymus is to generate and select a repertoire of T cells that will protect the body from infection. As thymocytes develop, an enormous diversity of T-cell receptors is generated by a random process (see Chapter 9) that produces some T cells with receptors capable of recognizing antigen-MHC complexes. However, most of the T-cell receptors produced by this random process are incapable of recognizing antigen-MHC complexes and a small portion react with combinations of self antigen-MHC complexes. Using mechanisms that are discussed in Chapter 10, the thymus induces the death of those T cells that cannot recognize antigen-MHC complexes and those that react with self-antigen–MHC and pose a danger of causing autoimmune disease. More than 95% of all thymocytes die by apoptosis in the thymus without ever reaching maturity.

THE THYMUS AND IMMUNE FUNCTION The role of the thymus in immune function can be studied in mice by examining the effects of neonatal thymectomy, a procedure in which the thymus is surgically removed from newborn mice. These thymectomized mice show a dramatic decrease in circulating lymphocytes of the T-cell lineage and an absence of cell-mediated immunity. Other evidence of the importance of the thymus comes from studies of a congenital birth defect in humans (**DiGeorge's syndrome**) and in certain mice (**nude mice**) in which the thymus fails to develop. In both cases, there is an absence of circulating T cells and of cell-mediated immunity and an increase in infectious disease.

Aging is accompanied by a decline in thymic function. This decline may play some role in the decline in immune function during aging in humans and mice. The thymus reaches its maximal size at puberty and then atrophies, with a significant decrease in both cortical and medullary cells and

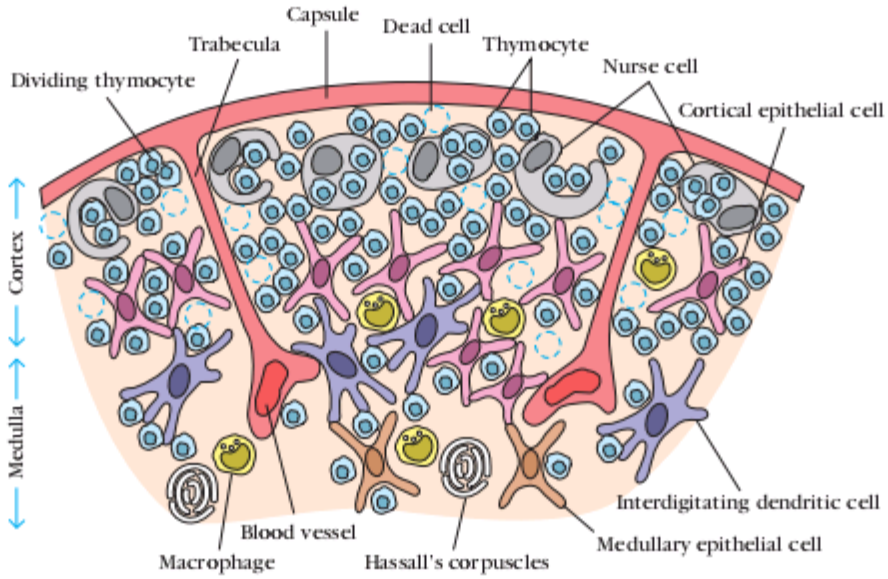

FIGURE 2-14 Diagrammatic cross section of a portion of the thymus, showing several lobules separated by connective tissue strands (trabeculae). The densely populated outer cortex is thought to contain many immature thymocytes (blue), which undergo rapid proliferation coupled with an enormous rate of cell death. Also present in the outer cortex are thymic nurse cells (gray), which are specialized epithelial cells with long membrane extensions that surround as many as 50 thymocytes. The medulla is sparsely populated and is thought to contain thymocytes that are more mature. During their stay within the thymus, thymocytes interact with various stromal cells, including cortical epithelial cells (light red), medullary epithelial cells (tan), interdigitating dendritic cells (purple), and macrophages (yellow). These cells produce thymic hormones and express high levels of class I and class II MHC molecules. Hassalls corpuscles, found in the medulla, contain concentric layers of degenerating epithelial cells. [*Adapted, with permission, from W. van Ewijk, 1991,* Annu. Rev. Immunol. ***9:591,*** *© 1991 by Annual Reviews.*]

an increase in the total fat content of the organ. Whereas the average weight of the thymus is 70 g in infants, its age-dependent involution leaves an organ with an average weight of only 3 g in the elderly (Figure 2-15).

A number of experiments have been designed to look at the effect of age on the immune function of the thymus. In one experiment, the thymus from a 1-day-old or 33-month-old mouse was grafted into thymectomized adults. (For most laboratory mice, 33 months is very old.) Mice receiving the newborn thymus graft showed a significantly larger improvement in immune function than mice receiving the 33-month-old thymus.

BONE MARROW

In humans and mice, bone marrow is the site of B-cell origin and development. Arising from lymphoid progenitors, immature B cells proliferate and differentiate within the bone marrow, and stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for development. Like thymic selection during T-cell maturation, a selection process within the bone marrow eliminates B cells with self-reactive antibody receptors. This process is explained in detail in Chapter 11. Bone marrow is not the site of B-cell development in all species. In birds, a lymphoid organ called the bursa of Fabricius, a lymphoid tissue associated with the gut, is the primary site of B-cell maturation. In mammals such as primates and rodents, there is no bursa and no single counterpart to it as a primary lymphoid organ. In cattle and sheep, the primary lymphoid tissue hosting the maturation, proliferation, and diversification of B cells early in gestation is the fetal spleen. Later in gestation, this function is assumed by a patch of tissue embedded

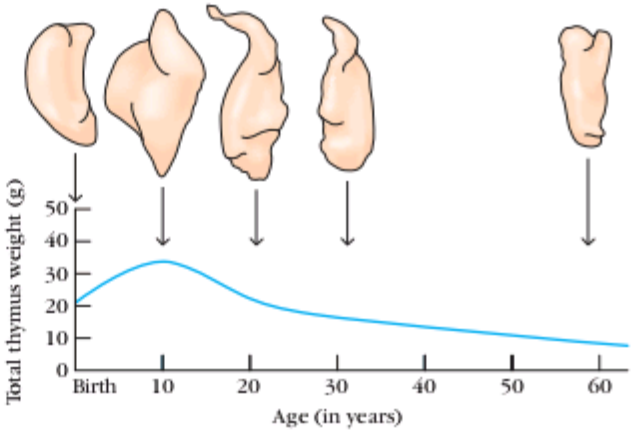

FIGURE 2-15 Changes in the thymus with age. The thymus decreases in size and cellularity after puberty.

in the wall of the intestine called the ileal Peyer's patch, which contains a large number ($>10^{10}$) B cells. The rabbit, too, uses gut-associated tissues such as the appendix as primary lymphoid tissue for important steps in the proliferation and diversification of B cells.

Lymphatic System

As blood circulates under pressure, its fluid component (**plasma**) seeps through the thin wall of the capillaries into the surrounding tissue. Much of this fluid, called **interstitial fluid,** returns to the blood through the capillary membranes. The remainder of the interstitial fluid, now called **lymph,** flows from the spaces in connective tissue into a network of tiny open lymphatic capillaries and then into a series of progressively larger collecting vessels called **lymphatic vessels** (Figure 2-16).

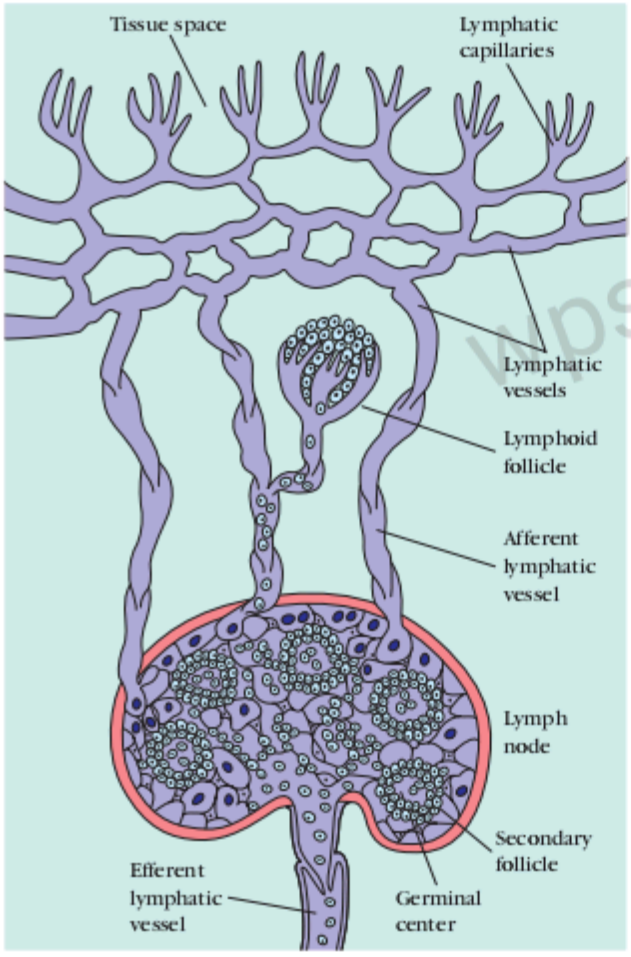

FIGURE 2-16 Lymphatic vessels. Small lymphatic capillaries opening into the tissue spaces pick up interstitial tissue fluid and carry it into progressively larger lymphatic vessels, which carry the fluid, now called lymph, into regional lymph nodes. As lymph leaves the nodes, it is carried through larger efferent lymphatic vessels, which eventually drain into the circulatory system at the thoracic duct or right lymph duct (see Figure 2-13).

The largest lymphatic vessel, the **thoracic duct,** empties into the left subclavian vein near the heart (see Figure 2-13). In this way, the lymphatic system captures fluid lost from the blood and returns it to the blood, thus ensuring steady-state levels of fluid within the circulatory system. The heart does not pump the lymph through the lymphatic system; instead the flow of lymph is achieved as the lymph vessels are squeezed by movements of the body's muscles. A series of one-way valves along the lymphatic vessels ensures that lymph flows only in one direction.

When a foreign antigen gains entrance to the tissues, it is picked up by the lymphatic system (which drains all the tissues of the body) and is carried to various organized lymphoid tissues such as lymph nodes, which trap the foreign antigen. As lymph passes from the tissues to lymphatic vessels, it becomes progressively enriched in lymphocytes. Thus, the lymphatic system also serves as a means of transporting lymphocytes and antigen from the connective tissues to organized lymphoid tissues where the lymphocytes may interact with the trapped antigen and undergo activation.

Secondary Lymphoid Organs

Various types of organized lymphoid tissues are located along the vessels of the lymphatic system. Some lymphoid tissue in the lung and lamina propria of the intestinal wall consists of diffuse collections of lymphocytes and macrophages. Other lymphoid tissue is organized into structures called lymphoid follicles, which consist of aggregates of lymphoid and nonlymphoid cells surrounded by a network of draining lymphatic capillaries. Until it is activated by antigen, a lymphoid follicle—called a **primary follicle**—comprises a network of follicular dendritic cells and small resting B cells. After an antigenic challenge, a primary follicle becomes a larger **secondary follicle**—a ring of concentrically packed B lymphocytes surrounding a center (the **germinal center**) in which one finds a focus of proliferating B lymphocytes and an area that contains nondividing B cells, and some helper T cells interspersed with macrophages and follicular dendritic cells (Figure 2-17).

Most antigen-activated B cells divide and differentiate into antibody-producing plasma cells in lymphoid follicles, but only a few B cells in the antigen-activated population find their way into germinal centers. Those that do undergo one or more rounds of cell division, during which the genes that encode their antibodies mutate at an unusually high rate. Following the period of division and mutation, there is a rigorous selection process in which more than 90% of these B cells die by apoptosis. In general, those B cells producing antibodies that bind antigen more strongly have a much better chance of surviving than do their weaker companions. The small number of B cells that survive the germinal center's rigorous selection differentiate into plasma cells or memory

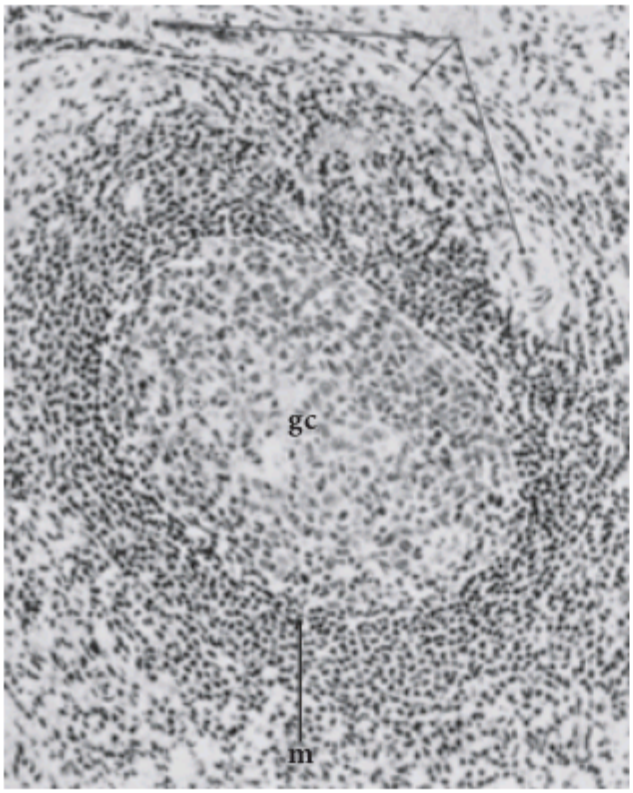

FIGURE 2-17 A secondary lymphoid follicle consisting of a large germinal center (gc) surrounded by a dense mantle (m) of small lymphocytes. [*From W. Bloom and D. W. Fawcett, 1975,* Textbook of Histology, *10th ed., © 1975 by W. B. Saunders Co.*]

cells and emerge. The process of B-cell proliferation, mutation, and selection in germinal centers is described more fully in Chapter 11.

Lymph nodes and the **spleen** are the most highly organized of the secondary lymphoid organs; they comprise not only lymphoid follicles, but additional distinct regions of T-cell and B-cell activity, and they are surrounded by a fibrous capsule. Less-organized lymphoid tissue, collectively called mucosal-associated lymphoid tissue (MALT), is found in various body sites. MALT includes Peyer's patches (in the small intestine), the tonsils, and the appendix, as well as numerous lymphoid follicles within the lamina propria of the intestines and in the mucous membranes lining the upper airways, bronchi, and genital tract.

LYMPH NODES

Lymph nodes are the sites where immune responses are mounted to antigens in lymph. They are encapsulated bean-shaped structures containing a reticular network packed with lymphocytes, macrophages, and dendritic cells. Clustered at junctions of the lymphatic vessels, lymph nodes are the first organized lymphoid structure to encounter antigens that enter the tissue spaces. As lymph percolates through a node, any particulate antigen that is brought in with the lymph will be trapped by the cellular network of phagocytic cells and dendritic cells (follicular and interdigitating). The overall architecture of a lymph node supports an ideal microenvironment for lymphocytes to effectively encounter and respond to trapped antigens.

Morphologically, a lymph node can be divided into three roughly concentric regions: the cortex, the paracortex, and the medulla, each of which supports a distinct microenvironment (Figure 2-18). The outermost layer, the **cortex,** contains lymphocytes (mostly B cells), macro-phages, and follicular dendritic cells arranged in primary follicles. After antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal center. In children with B-cell deficiencies, the cortex lacks primary follicles and germinal centers. Beneath the cortex is the **paracortex,** which is populated largely by T lymphocytes and also contains interdigitating dendritic cells thought to have migrated from tissues to the node. These interdigitating dendritic cells express high levels of class II MHC molecules, which are necessary for presenting antigen to T_H cells. Lymph nodes taken from neonatally thymectomized mice have unusually few cells in the paracortical region; the paracortex is therefore sometimes referred to as a **thymus-dependent area** in contrast to the cortex, which is a **thymus-independent area.** The innermost layer of a lymph node, the **medulla,** is more sparsely populated with lymphoid-lineage cells; of those present, many are plasma cells actively secreting antibody molecules.

As antigen is carried into a regional node by the lymph, it is trapped, processed, and presented together with class II MHC molecules by interdigitating dendritic cells in the paracortex, resulting in the activation of T_H cells. The initial activation of B cells is also thought to take place within the T-cell-rich paracortex. Once activated, T_H and B cells form small foci consisting largely of proliferating B cells at the edges of the paracortex. Some B cells within the foci differentiate into plasma cells secreting IgM and IgG. These foci reach maximum size within 4–6 days of antigen challenge. Within 4–7 days of antigen challenge, a few B cells and T_H cells migrate to the primary follicles of the cortex. It is not known what causes this migration. Within a primary follicle, cellular interactions between follicular dendritic cells, B cells, and T_H cells take place, leading to development of a secondary follicle with a central germinal center. Some of the plasma cells generated in the germinal center move to the medullary areas of the lymph node, and many migrate to bone marrow.

Afferent lymphatic vessels pierce the capsule of a lymph node at numerous sites and empty lymph into the subcapsular sinus (see Figure 2-18b). Lymph coming from the tissues percolates slowly inward through the cortex, paracortex, and medulla, allowing phagocytic cells and dendritic cells to trap any bacteria or particulate material (e.g., antigen-antibody complexes) carried by the lymph. After infection or the

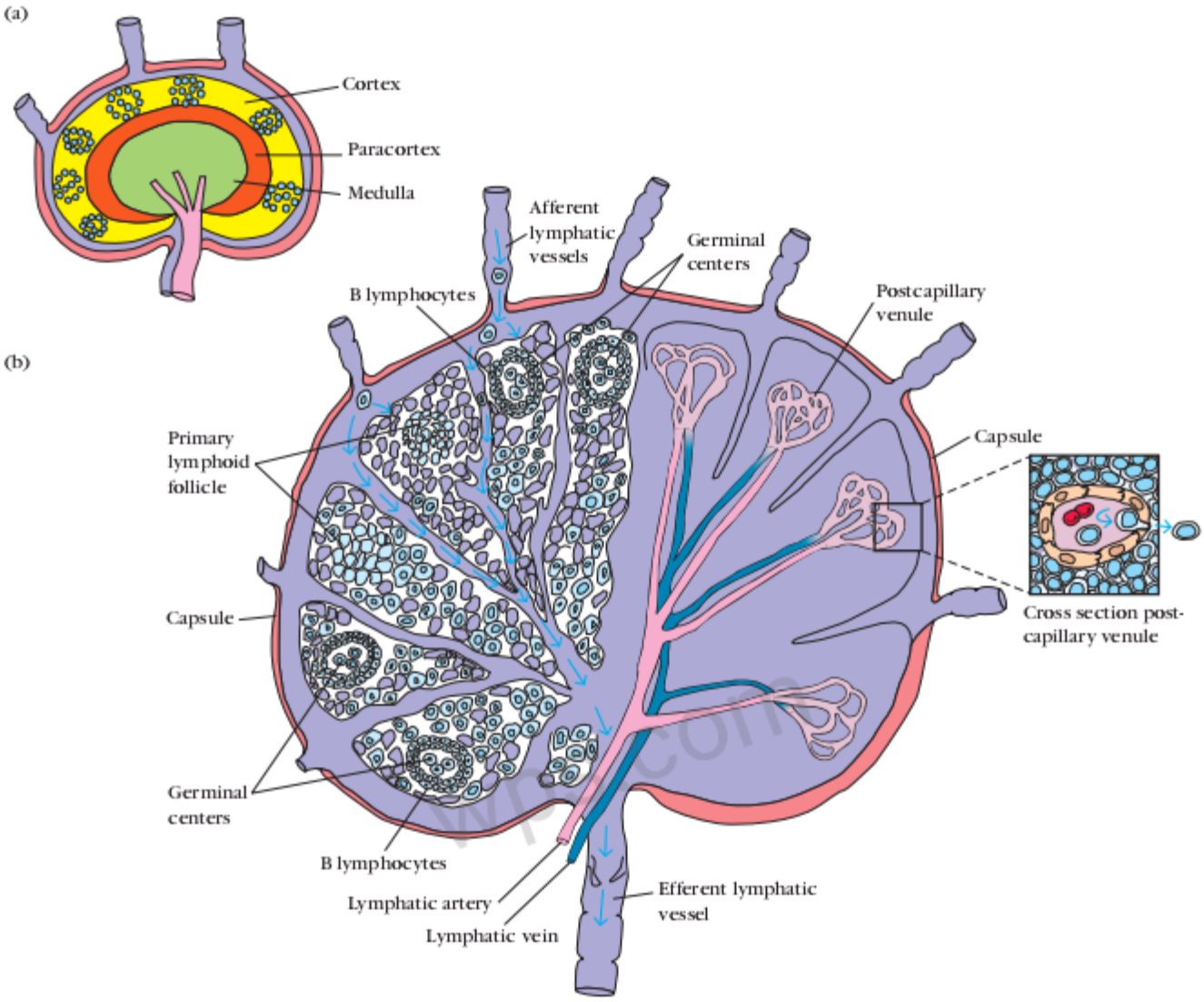

FIGURE 2-18 Structure of a lymph node. (a) The three layers of a lymph node support distinct microenvironments. (b) The left side depicts the arrangement of reticulum and lymphocytes within the various regions of a lymph node. Macrophages and dendritic cells, which trap antigen, are present in the cortex and paracortex. T_H cells are concentrated in the paracortex; B cells are located primarily in the cortex, within follicles and germinal centers. The medulla is populated largely by antibody-producing plasma cells. Lymphocytes circulating in the lymph are carried into the node by afferent lymphatic vessels; they either enter the reticular matrix of the node or pass through it and leave by the efferent lymphatic vessel. The right side of (b) depicts the lymphatic artery and vein and the postcapillary venules. Lymphocytes in the circulation can pass into the node from the postcapillary venules by a process called extravasation (*inset*).

introduction of other antigens into the body, the lymph leaving a node through its single efferent lymphatic vessel is enriched with antibodies newly secreted by medullary plasma cells and also has a fiftyfold higher concentration of lymphocytes than the afferent lymph.

The increase in lymphocytes in lymph leaving a node is due in part to lymphocyte proliferation within the node in response to antigen. Most of the increase, however, represents blood-borne lymphocytes that migrate into the node by passing between specialized endothelial cells that line the **postcapillary venules** of the node. Estimates are that 25% of the lymphocytes leaving a lymph node have migrated across this endothelial layer and entered the node from the blood. Because antigenic stimulation within a node can increase this migration tenfold, the concentration of lymphocytes in a node that is actively responding can increase greatly, and the node swells visibly. Factors released in lymph nodes during antigen stimulation are thought to facilitate this increased migration.

SPLEEN

The spleen plays a major role in mounting immune responses to antigens in the blood stream. It is a large, ovoid secondary lymphoid organ situated high in the left abdominal cavity. While lymph nodes are specialized for trapping antigen from local tissues, the spleen specializes in filtering blood and trapping blood-borne antigens; thus, it can respond to systemic infections. Unlike the lymph nodes, the spleen is not supplied by lymphatic vessels. Instead, blood-borne antigens and lymphocytes are carried into the spleen through the splenic artery. Experiments with radioactively labeled lymphocytes show that more recirculating lymphocytes pass daily through the spleen than through all the lymph nodes combined.

The spleen is surrounded by a capsule that extends a number of projections (trabeculae) into the interior to form a compartmentalized structure. The compartments are of two types, the red pulp and white pulp, which are separated by a diffuse marginal zone (Figure 2-19). The splenic **red pulp** consists of a network of sinusoids populated by macrophages and numerous red blood cells (erythrocytes) and few lymphocytes; it is the site where old and defective red blood cells are destroyed and removed. Many of the macrophages within the red pulp contain engulfed red blood cells or iron pigments from degraded hemoglobin. The splenic **white pulp** surrounds the branches of the splenic artery, forming a **periarteriolar lymphoid sheath (PALS)** populated mainly by T lymphocytes. Primary lymphoid follicles are attached to the

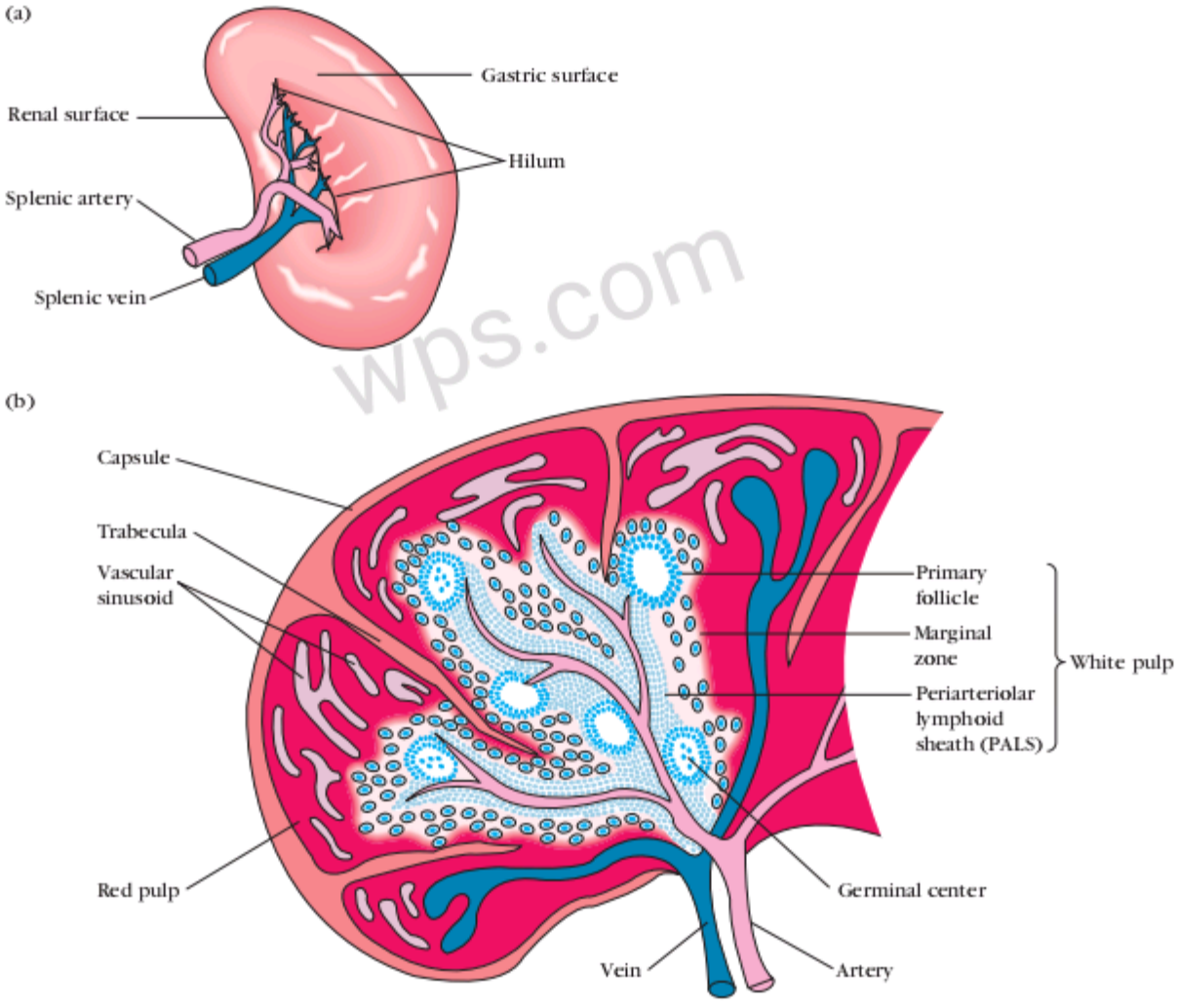

FIGURE 2-19 Structure of the spleen. (a) The spleen, which is about 5 inches long in adults, is the largest secondary lymphoid organ. It is specialized for trapping blood-borne antigens. (b) Diagrammatic cross section of the spleen. The splenic artery pierces the capsule and divides into progressively smaller arterioles, ending in vascular sinusoids that drain back into the splenic vein. The erythrocyte-filled red pulp surrounds the sinusoids. The white pulp forms a sleeve, the periarteriolar lymphoid sheath (PALS), around the arterioles; this sheath contains numerous T cells. Closely associated with the PALS is the marginal zone, an area rich in B cells that contains lymphoid follicles that can develop into secondary follicles containing germinal centers.

PALS. These follicles are rich in B cells and some of them contain germinal centers. The **marginal zone,** located peripheral to the PALS, is populated by lymphocytes and macrophages.

Blood-borne antigens and lymphocytes enter the spleen through the splenic artery, which empties into the marginal zone. In the marginal zone, antigen is trapped by interdigitating dendritic cells, which carry it to the PALS. Lymphocytes in the blood also enter sinuses in the marginal zone and migrate to the PALS.

The initial activation of B and T cells takes place in the T-cell-rich PALS. Here interdigitating dendritic cells capture antigen and present it combined with class II MHC molecules to T_H cells. Once activated, these T_H cells can then activate B cells. The activated B cells, together with some T_H cells, then migrate to primary follicles in the marginal zone. Upon antigenic challenge, these primary follicles develop into characteristic secondary follicles containing germinal centers (like those in the lymph nodes), where rapidly dividing B cells (centroblasts) and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes.

The effects of splenectomy on the immune response depends on the age at which the spleen is removed. In children, splenectomy often leads to an increased incidence of bacterial sepsis caused primarily by *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae.* Splenectomy in adults has less adverse effects, although it leads to some increase in blood-borne bacterial infections (**bacteremia**).

MUCOSAL-ASSOCIATED LYMPHOID TISSUE

The mucous membranes lining the digestive, respiratory, and urogenital systems have a combined surface area of about 400 m^2 (nearly the size of a basketball court) and are the major sites of entry for most pathogens. These vulnerable membrane surfaces are defended by a group of organized lymphoid tissues mentioned earlier and known collectively as **mucosal-associated lymphoid tissue (MALT).** Structurally, these tissues range from loose, barely organized clusters of lymphoid cells in the lamina propria of intestinal villi to well-organized structures such as the familiar tonsils and appendix, as well as Peyer's patches, which are found within the submucosal layer of the intestinal lining. The functional importance of MALT in the body's defense is attested to by its large population of antibody-producing plasma cells, whose number far exceeds that of plasma cells in the spleen, lymph nodes, and bone marrow combined.

The **tonsils** are found in three locations: lingual at the base of the tongue; palatine at the sides of the back of the mouth; and pharyngeal (adenoids) in the roof of the nasopharynx (Figure 2-20). All three tonsil groups are nodular structures consisting of a meshwork of reticular cells and fibers interspersed with lymphocytes, macrophages, granulocytes, and mast cells. The B cells are organized into follicles and germinal centers; the latter are surrounded by regions showing T-cell activity. The tonsils defend against antigens entering through the nasal and oral epithelial routes.

The best studied of the mucous membranes is the one that lines the gastrointestinal tract. This tissue, like that of the respiratory and urogenital tracts, has the capacity to endocytose antigen from the lumen. Immune reactions are initiated against pathogens and antibody can be generated and exported to the lumen to combat the invading organisms. As shown in Figures 2-21 and 2-22, lymphoid cells are found in various regions within this tissue. The outer mucosal epithe-

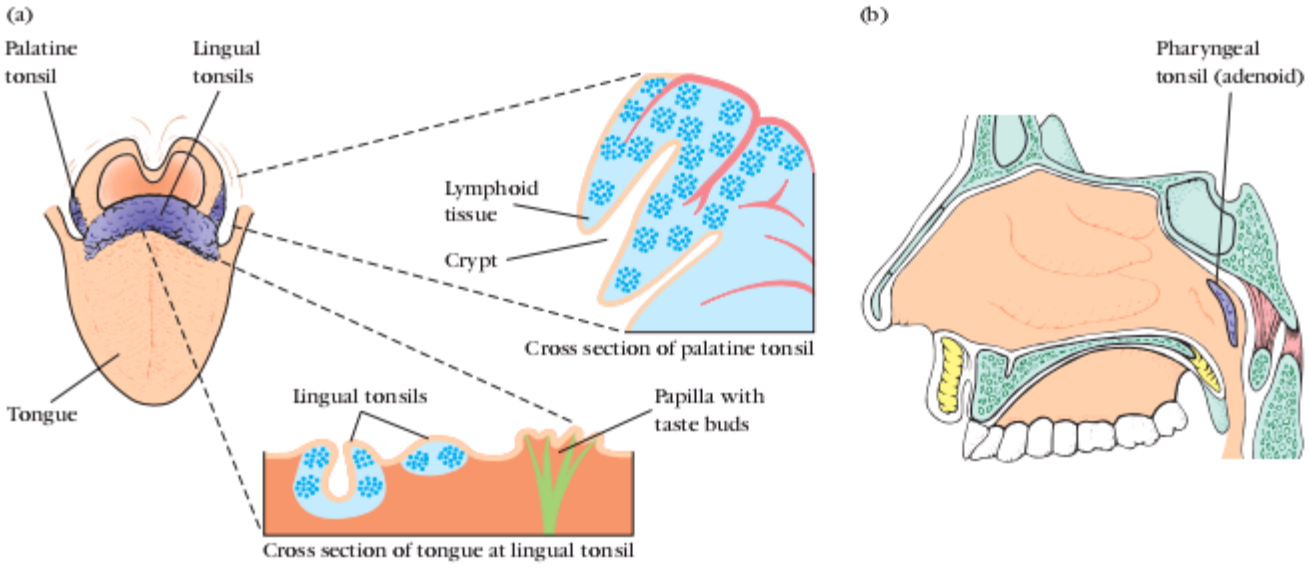

FIGURE 2-20 Three types of tonsils. (a) The position and internal features of the palatine and lingual tonsils; (b) a view of the position of the nasopharyngeal tonsils (adenoids). [*Part b adapted from J. Klein, 1982,* Immunology, The Science of Self-Nonself Discrimination, *© 1982 by John Wiley and Sons, Inc.*]

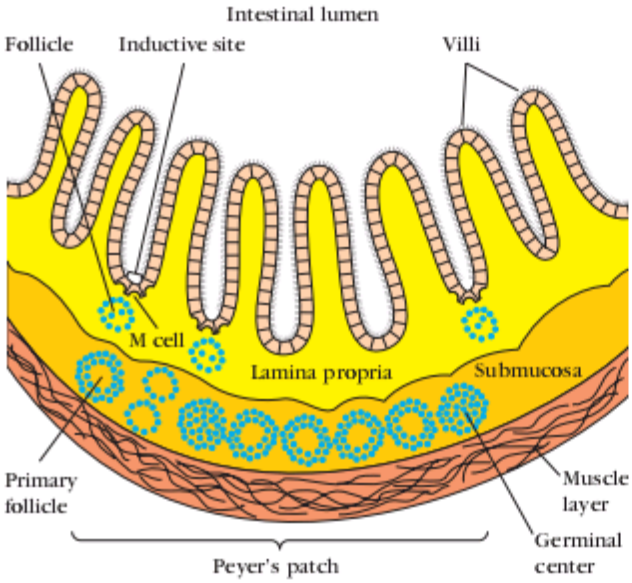

FIGURE 2-21 Gross-sectional diagram of the mucous membrane lining the intestine showing a nodule of lymphoid follicles that constitutes a Peyer's patch in the submucosa. The intestinal lamina propria contains loose clusters of lymphoid cells and diffuse follicles.

lial layer contains so-called **intraepithelial lymphocytes (IELs).** Many of these lymphocytes are T cells that express unusual receptors (γδT-cell receptors, or γδ TCRs), which exhibit limited diversity for antigen. Although this population of T cells is well situated to encounter antigens that enter through the intestinal mucous epithelium, their actual function remains largely unknown The lamina propria, which lies under the epithelial layer, contains large numbers of B cells, plasma cells, activated T_H cells, and macrophages in loose clusters. Histologic sections have revealed more than 15,000 lymphoid follicles within the intestinal lamina propria of a healthy child. The submucosal layer beneath the lamina propria contains Peyer's patches, nodules of 30–40 lymphoid follicles. Like lymphoid follicles in other sites, those that compose Peyer's patches can develop into secondary follicles with germinal centers.

The epithelial cells of mucous membranes play an important role in promoting the immune response by delivering small samples of foreign antigen from the lumina of the respiratory, digestive, and urogenital tracts to the underlying mucosal-associated lymphoid tissue. This antigen transport is carried out by specialized **M cells.** The structure of the M cell is striking: these are flattened epithelial cells lacking the microvilli that characterize the rest of the mucous epithelium. In addition, M cells have a deep invagination, or pocket, in the basolateral plasma membrane; this pocket is filled with a cluster of B cells, T cells, and macrophages (Figure 2-22a). Luminal antigens are endocytosed into vesicles that are transported from the luminal membrane to the underlying pocket membrane. The vesicles then fuse with the pocket membrane, delivering the potentially response-activating antigens to the clusters of lymphocytes contained within the pocket.

M cells are located in so-called **inductive sites**—small regions of a mucous membrane that lie over organized lymphoid follicles (Figure 2-22b). Antigens transported across the mucous membrane by M cells can activate B cells within

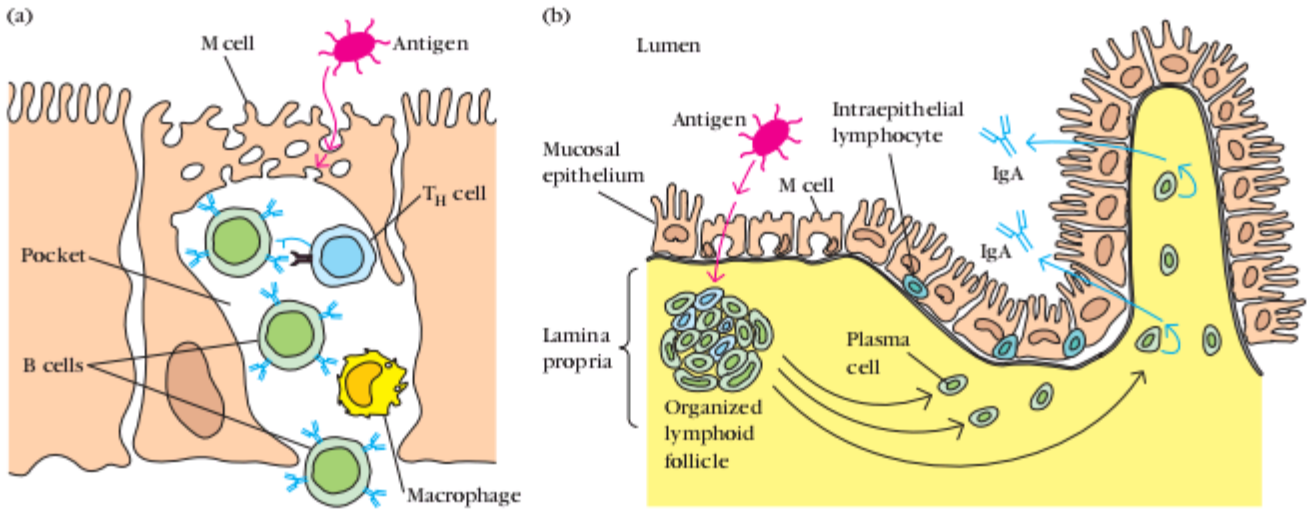

FIGURE 2-22 Structure of M cells and production of IgA at inductive sites. (a) M cells, located in mucous membranes, endocytose antigen from the lumen of the digestive, respiratory, and urogenital tracts. The antigen is transported across the cell and released into the large basolateral pocket. (b) Antigen transported across the epithelial layer by M cells at an inductive site activates B cells in the underlying lymphoid follicles. The activated B cells differentiate into IgA-producing plasma cells, which migrate along the submucosa. The outer mucosal epithelial layer contains intraepithelial lymphocytes, of which many are $CD8^+$ T cells that express γδ TCRs with limited receptor diversity for antigen.

these lymphoid follicles. The activated B cells differentiate into plasma cells, which leave the follicles and secrete the IgA class of antibodies. These antibodies then are transported across the epithelial cells and released as **secretory IgA** into the lumen, where they can interact with antigens.

As described in Chapter 1, mucous membranes are an effective barrier to the entrance of most pathogens, which thereby contributes to nonspecific immunity. One reason for this is that the mucosal epithelial cells are cemented to one another by tight junctions that make it difficult for pathogens to penetrate. Interestingly, some enteric pathogens, including both bacteria and viruses, have exploited the M cell as an entry route through the mucous-membrane barrier. In some cases, the pathogen is internalized by the M cell and transported into the pocket. In other cases, the pathogen binds to the M cell and disrupts the cell, thus allowing entry of the pathogen. Among the pathogens that use M cells in these ways are several invasive *Salmonella* species, *Vibrio cholerae*, and the polio virus.

Cutaneous-Associated Lymphoid Tissue

The skin is an important anatomic barrier to the external environment, and its large surface area makes this tissue important in nonspecific (innate) defenses. The epidermal (outer) layer of the skin is composed largely of specialized epithelial cells called keratinocytes. These cells secrete a number of cytokines that may function to induce a local inflammatory reaction. In addition, keratinocytes can be induced to express class II MHC molecules and may function as antigen-presenting cells. Scattered among the epithelial-cell matrix of the epidermis are Langerhans cells, a type of dendritic cell, which internalize antigen by phagocytosis or endocytosis. The Langerhans cells then migrate from the epidermis to regional lymph nodes, where they differentiate into interdigitating dendritic cells. These cells express high levels of class II MHC molecules and function as potent activators of naive T_H cells.

The epidermis also contains so-called *intraepidermal lymphocytes*. These are similar to the intraepithelial lymphocytes of MALT in that most of them are $CD8^+$ T cells, many of which express γδ T-cell receptors, which have limited diversity for antigen. These intraepidermal T cells are well situated to encounter antigens that enter through the skin and some immunologists believe that they may play a role in combating antigens that enter through the skin. The underlying dermal layer of the skin contains scattered $CD4^+$ and $CD8^+$ T cells and macrophages. Most of these dermal T cells were either previously activated cells or are memory cells.

Systemic Function of the Immune System

The many different cells, organs, and tissues of the immune system are dispersed throughout the body, yet the various components communicate and collaborate to produce an effective response to an infection. An infection that begins in one area of the body initiates processes that eventually involve cells, organs, and tissues distant from the site of pathogen invasion. Consider what happens when the skin is broken, allowing bacteria to enter the body and initiate infection.

The tissue damage associated with the injury and infection results in an inflammatory response that causes increased blood flow, vasodilation, and an increase in capillary permeability. Chemotactic signals are generated that can cause phagocytes and lymphocytes to leave the blood stream and enter the affected area. Factors generated during these early stages of the infection stimulate the capacity of the adaptive immune system to respond. Langerhans cells (dendritic cells found throughout the epithelial layers of the skin and the respiratory, gastrointestinal, urinary, and genital tracts) can capture antigens from invading pathogens and migrate into a nearby lymphatic vessel, where the flow of lymph carries them to nearby lymph nodes. In the lymph nodes these class II MHC–bearing cells can become members of the interdigitating dendritic-cell population and initiate adaptive immune responses by presenting antigen to T_H cells. The recognition of antigen by T_H cells can have important consequences, including the activation and proliferation of T_H cells within the node as the T_H cells recognize the antigen, and the secretion by the activated T cells of factors that support T-cell–dependent antibody production by B cells that may already have been activated by antigen delivered to the lymph node by lymph. The antigen-stimulated T_H cells also release chemotactic factors that cause lymphocytes to leave the blood circulation and enter the lymph node through the endothelium of the postcapillary venules. Lymphocytes that respond to the antigen are retained in the lymph node for 48 hours or so as they undergo activation and proliferation before their release via the node's efferent lymphatic vessel.

Once in the lymph, the newly released activated lymphocytes can enter the bloodstream via the subclavian vein. Eventually, the circulation carries them to blood vessels near the site of the infection, where the inflammatory process makes the vascular endothelium of the nearby blood vessels more adherent for activated T cells and other leukocytes (see Chapter 15). Chemotactic factors that attract lymphocytes, macrophages, and neutrophils are also generated during the inflammatory process, promoting leukocyte adherence to nearby vascular epithelium and leading leukocytes to the site of the infection. Later in the course of the response, pathogen-specific antibodies produced in the node are also carried to the bloodstream. Inflammation aids the delivery of the anti-pathogen antibody by promoting increased vascular permeability, which increases the flow of antibody-containing plasma from the blood circulation to inflamed tissue. The result of this network of interactions among diffusible molecules, cells, organs, the lymphatic system, and the circulatory system is an effective and focused immune response to an infection.

Lymphoid Cells and Organs—Evolutionary Comparisons

While innate systems of immunity are seen in invertebrates and even in plants, the evolution of lymphoid cells and organs evolved only in the phylum Vertebrata. Consequently, adaptive immunity, which is mediated by antibodies and T cells, is only seen in this phylum. However, as shown in Figure 2-23, the kinds of lymphoid tissues seen in different orders of vertebrates differ.

As one considers the spectrum from the earliest vertebrates, the jawless fishes (Agnatha), to the birds and mammals, evolution has added organs and tissues with immune

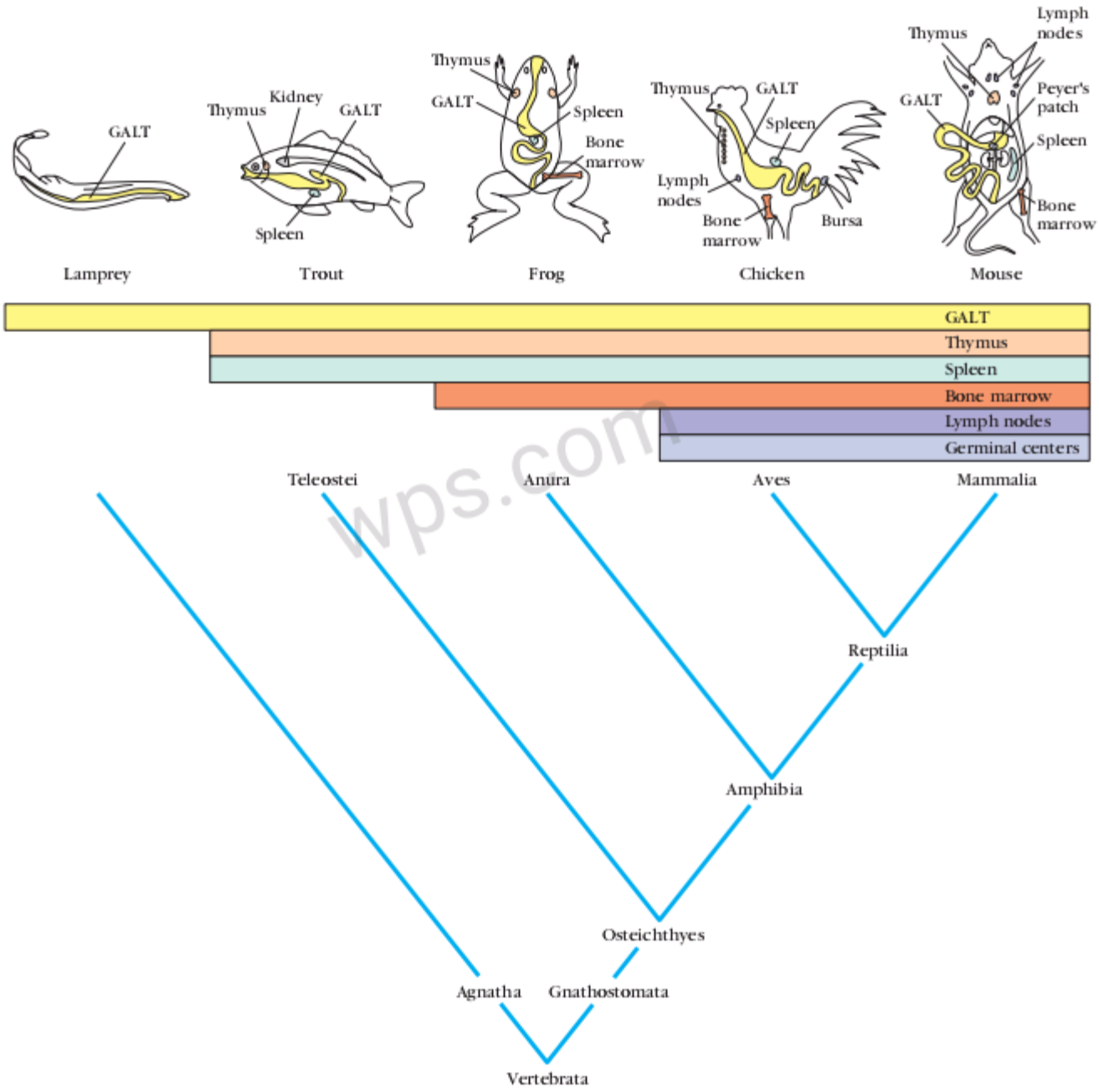

FIGURE 2-23 Evolutionary distribution of lymphoid tissues. The presence and location of lymphoid tissues in several major orders of vertebrates are shown. Although they are not shown in the diagram, cartilaginous fish such as sharks and rays have GALT, thymus, and a spleen. Reptiles also have GALT, thymus, and spleen and they also may have lymph nodes that participate in immunological reactions. Whether bone marrow is involved in the generation of lymphocytes in reptiles is under investigation. [*Adapted from Dupasquier and M. Flajnik, 1999. In* Fundamental Immunology *4th ed., W. E. Paul, ed., Lippincott-Raven, Philadelphia.*]