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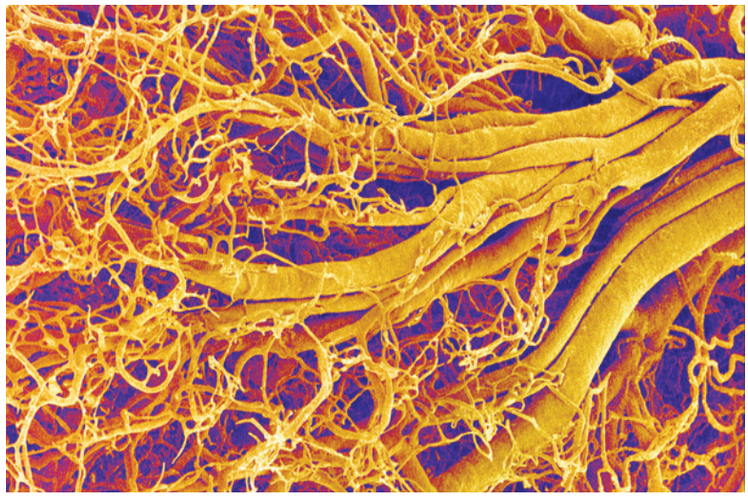
Cells, Organs, and Microenvironments of the Immune System



Scanning electron micrograph of blood vessels in a lymph node. *[Susumu Nishinaga/Science Source.]*

Learning Objectives

After reading this chapter, you should be able to:

1. Describe the types of blood cells that make up the immune system and outline the main events that occur during hematopoiesis, the process that gives rise to immune cells.
2. Identify the primary, secondary, and tertiary immune organs in vertebrates and describe their function.
3. Recognize and describe the microenvironments where immune cells mature and the immune response develops.
4. Identify several experimental approaches used to understand how blood cells and immune responses develop.

A successful immune response to a pathogen depends on finely choreographed interactions among diverse cell types (see Figure 1-7): innate immune cells that mount the first line of defense against pathogen, antigen-presenting cells that communicate the infection to lymphoid cells, which coordinate the adaptive response and generate the memory cells that prevent future infections. The coordination required for a full immune response is made possible by the specialized anatomy and microanatomy of the immune system, which is dispersed throughout the body and organizes cells in time and space. **Primary lymphoid organs**—including the bone marrow and the thymus—are sites where immune cells develop from immature precursors. **Secondary lymphoid organs**—including the spleen, lymph nodes, and specialized sites in the gut and other mucosal tissues—are sites where the mature antigen-specific lymphocytes first encounter antigen and begin their differentiation into effector and memory cells. Two circulatory systems—blood and lymphatic vessels—connect these organs, uniting them into a functional whole.

Remarkably, all mature blood cells, including red blood cells, granulocytes, macrophages, dendritic cells, and lymphocytes, arise from a single cell type, the **hematopoietic stem cell (HSC)** (**Figure 2-1**). We begin this chapter with a description of **hematopoiesis**, the process by which HSCs differentiate into mature blood cells. We describe the features and function of the various cell types that arise from HSCs and then discuss the anatomy and microanatomy of the major primary lymphoid organs where hematopoiesis takes place. We feature the lymph nodes and the spleen in our

Key Terms

- Hematopoiesis
- Hematopoietic stem cell (HSC)
- Myeloid lineage cells
- Lymphoid lineage cells
- Primary lymphoid organs
  - Bone marrow
  - Thymus
- Secondary lymphoid organs
  - Lymph nodes
  - Spleen
  - Barrier tissues (MALT and skin)
- Lymphatic system
- Tertiary lymphoid tissue
- T-cell zone
- B-cell follicle
- Germinal centers
- Fibroblastic reticular cell conduit (FRCC) system
- Follicular dendritic cells (FDCs)

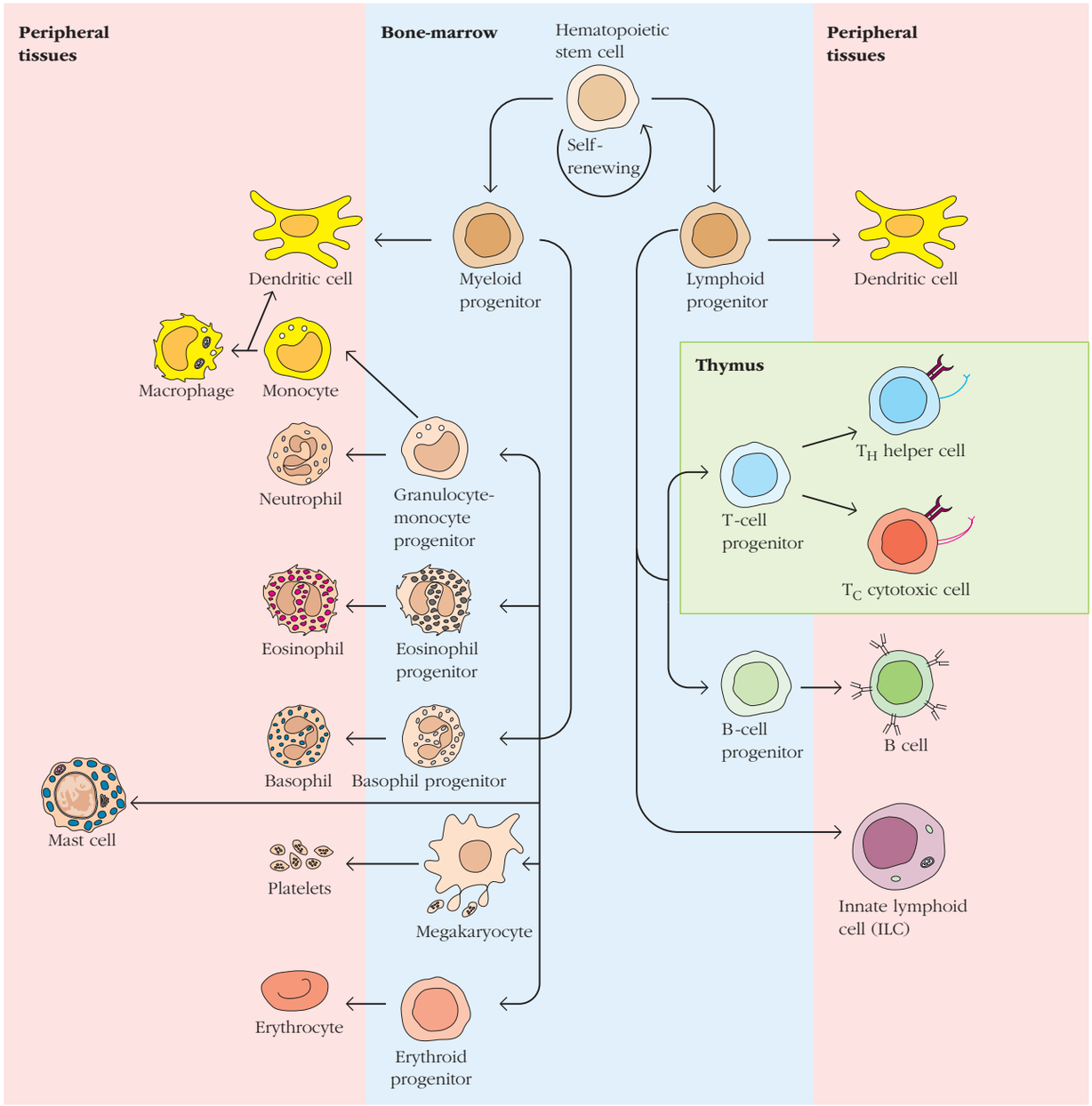


FIGURE 2-1 Hematopoiesis. Self-renewing hematopoietic stem cells give rise to lymphoid and myeloid progenitors. Most immune cells mature in the bone marrow and then travel to peripheral organs via the blood. Some, including mast cells and macrophages, undergo further maturation outside the bone marrow. T cells develop to maturity in the thymus.

description of secondary lymphoid organs. The secondary lymphoid tissue in the distinctive mucosal immune system is described in Chapter 13.

Four focused discussions are also included in this chapter. In two Classic Experiment Boxes, we describe the discovery of a second thymus and the history behind the identification of hematopoietic stem cells. In a Clinical Focus Box, we discuss the clinical use and promise of hematopoietic stem cells, and finally, in an Evolution Box, we describe some intriguing variations in the anatomy of the immune system among our vertebrate relatives.

Hematopoiesis and Cells of the Immune System

Stem cells are defined by two capacities: (1) the ability to regenerate or "self-renew" and (2) the ability to differentiate into diverse cell types. Embryonic stem cells have the capacity to generate almost every specialized cell type in an organism (in other words, they are *pluripotent*). Adult stem cells, in contrast, have the capacity to give rise to the diverse cell types that specify a particular tissue (they are *multipotent*). Multiple adult organs harbor stem cells that can give rise to cells specific for that tissue (*tissue-specific stem cells*). The HSC was the first tissue-specific stem cell identified and is the source of all of our red blood cells (erythroid cells) and white blood cells (leukocytes).

Hematopoietic Stem Cells Differentiate into All Red and White Blood Cells

HSCs originate in fetal tissues and reside primarily in the bone marrow of adult vertebrates. A small number can be found in the adult spleen and liver. Regardless of where they reside, HSCs are a rare subset—less than one HSC is present per 5×10^4 cells in the bone marrow. Their numbers are strictly controlled by a balance of cell division, death, and differentiation. Their development is tightly regulated by signals they receive in the microenvironments of primary lymphoid organs.

Under conditions when the immune system is not being challenged by a pathogen (steady state or **homeostatic** conditions), most HSCs are quiescent; only a small number divide, generating daughter cells. Some daughter cells retain the stem-cell characteristics of the mother cell—that is, they remain self-renewing and are able to give rise to all blood cell types. Other daughter cells differentiate into *progenitor cells* that have limited self-renewal capacity and become progressively more committed to a particular blood cell lineage. As an organism ages, the number of HSCs decreases, demonstrating that there are limits to an HSC's self-renewal potential.

When there is an increased demand for hematopoiesis, for example, during an infection or after chemotherapy, 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). Such irradiated mice die within 10 days unless they are infused with normal bone marrow cells from a genetically identical mouse. Although a normal mouse has 3×10^8 bone marrow cells, infusion of fewer than 10^4 bone marrow cells from a donor is sufficient to completely restore the hematopoietic system. Our ability to identify and purify this tiny subpopulation has improved considerably, and in theory we can rescue the immune systems of irradiated animals with just a few purified stem cells, which give rise to progenitors that proliferate rapidly and repopulate the blood system.

Because of their rarity, investigators initially found it very difficult to identify and isolate HSCs. **Classic Experiment Box 2-1** describes experimental approaches that led to the first successful isolation of HSCs. Briefly, these efforts featured clever process-of-elimination strategies. Investigators reasoned that undifferentiated HSCs would not express surface markers specific for mature cells from the multiple blood lineages ("Lin" markers). They used several approaches to eliminate cells in the bone marrow that did express these markers (Lin^+ cells) and then examined the remaining (Lin^-) population for its potential to continually give rise to all blood cells over the long term. Other investigators took advantage of two technological developments that revolutionized immunological research—monoclonal antibodies and flow cytometry (see Chapter 20)—and identified surface proteins, including CD34, Sca-1, and c-Kit, that were expressed by the rare HSC population and allowed them to be isolated directly.

We now recognize several different types of Lin– Sca-1^+c-Kit^+ (LSK) HSCs, which vary in their capacity for self-renewal and their ability to give rise to all blood cell populations (pluripotency). *Long-term HSCs* (*LT-HSCs*) are the most quiescent and retain pluripotency throughout the life of an organism. These give rise to *short-term HSCs* (*ST-HSCs*), which are also predominantly quiescent but divide more frequently and have limited self-renewal capacity. In addition to being a useful marker for identifying HSCs, c-Kit is a receptor for the cytokine SCF, which promotes the development of ***m*ulti*p*otent *p*rogenitors (MPPs)**; these cells have a much more limited ability to self-renew, but proliferate rapidly and can give rise to both lymphoid and myeloid cell lineages.

Key Concepts:

- All red and white blood cells develop from pluripotent HSCs during a highly regulated process called *hematopoiesis*. In the adult vertebrate, hematopoiesis occurs primarily in the bone marrow, a primary lymphoid organ that supports both the self-renewal of stem cells and their differentiation into multiple blood cell types.
- The HSC is a rare cell type that is self-renewing and multipotent. HSCs have the capacity to differentiate and replace blood cells rapidly. First isolated by negative selection techniques that enriched for undifferentiated stem cells, they are now isolated by high-powered sorting techniques.
- HSCs include multiple subpopulations that vary in their quiescence and capacity to self-renew. Long-term HSCs are the most quiescent and long-lived. They give rise to short-term HSCs, which can develop into more proliferative MPPs, which give rise to lymphoid and myeloid cell types.

CLASSIC EXPERIMENT BOX 2-1

Isolating Hematopoietic Stem Cells

By the 1960s researchers knew that HSCs existed and were a rare population in the bone marrow. However, they did not have the technology or knowledge required to isolate HSCs for clinical study and applications. How do you find something that is very rare, whose only distinctive feature is its function—its ability to give rise to all blood cells? Investigators adopted clever strategies to find the elusive HSC and owed a great deal to rapidly evolving technologies, including the advent of monoclonal antibodies and flow cytometry (see Chapter 20).

Investigators recognized that HSCs were unlikely to express proteins specific for mature blood cells. Using monoclonal antibodies raised against multiple mature cells, they trapped and removed mature cells from bone marrow cell suspensions. They started with a process called *panning* (**Figure 1**), in which the heterogeneous pool of bone marrow cells was incubated with antibodies bound to plastic. Mature cells stuck to the antibodies and cells that did not express these surface markers were gently dislodged and collected. Investigators showed that cells that did not stick were enriched for stem cells by several thousand-fold with this approach. One of the first images of human stem cells isolated by panning is shown in Figure 1. This negative selection strategy remains very useful today, and stem cells enriched by removing mature blood cells are referred to as "Lin$^-$" cells, reflecting their lack of lineage-specific surface markers.

Once investigators were able to identify surface proteins specifically expressed by HSCs, such as CD34, they could use techniques to positively select cells from heterogeneous bone marrow cell populations. The flow cytometer offered the most powerful way to pull out a rare population from a diverse group of cells. This machine, invented by the Herzenberg laboratory and its interdisciplinary team of inventors, has revolutionized immunology and clinical medicine. In a nutshell, it is a machine that can identify, separate, and recover individual cells on the basis of their unique protein and/or gene expression patterns. These patterns are revealed by fluorescent reagents, including antibodies. Irv Weissman and colleagues took advantage of each of these advances and, using a combination of positive and negative selection, developed an efficient approach to isolate HSCs (**Figure 2**).

At present, investigators agree that HSCs are enriched among cells that bear no mature (lineage-specific) markers, but express both the surface proteins Sca-1 and c-Kit. These are referred to as Lin$^-$Sca-1$^+$c-Kit$^+$ or LSK cells. Even this subgroup, which represents less than 1% of bone marrow cells, is phenotypically and functionally heterogeneous and investigators routinely evaluate 10 or more additional protein markers to sort through the multiple types of cells that have stem cell capacities. This breakthrough is only one of many that emerge from a combination of technological and experimental creativity, a synergy that continues to drive experimental advances.

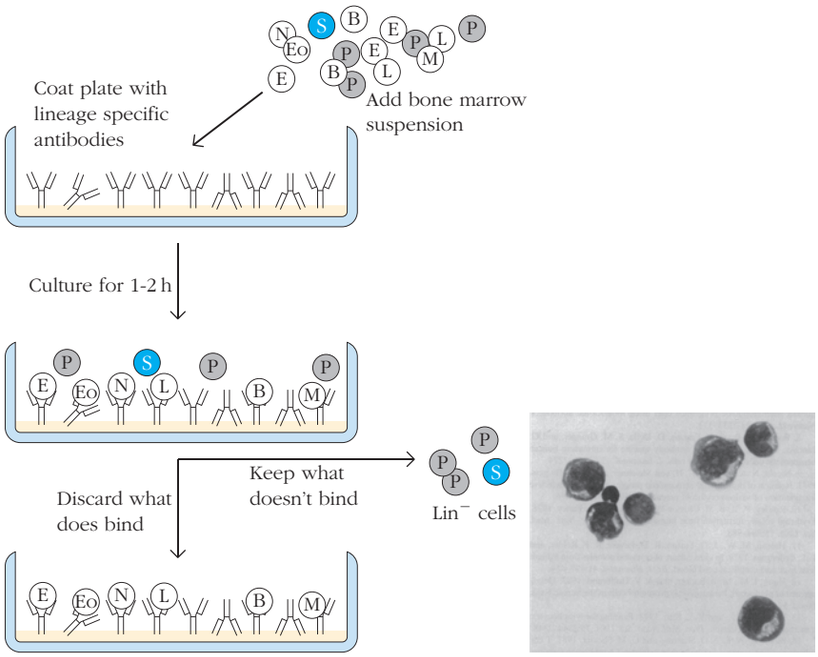


FIGURE 1 **Panning for stem cells.** Early approaches to isolate hematopoietic stem cells (HSCs) took advantage of antibodies that were raised against mature blood cells and a process called *panning*. Briefly, investigators layered a suspension of bone marrow cells onto plastic plates coated with antibodies that would bind multiple mature ("lineage-positive" [Lin$^+$]) blood cells. Cells that did not stick were therefore enriched for HSCs (the "lineage-negative" [Lin$^-$] cells desired). One of the first images of HSCs isolated in this way is shown. Abbreviations: S = stem cell; P = progenitor cell; M = monocyte; B = basophil; N = neutrophil; Eo = eosinophil; L = lymphocyte; E = erythrocyte. *[Republished with permission of The American Society for Clinical Investigation, from Emerson, S.G., et al. from "Purification and demonstration of fetal hematopoietic progenitors and demonstration of recombinant multipotential colony-stimulating activity," J. Clin. Invest., Sept. 1985* ***76****: 1286–1290, Figure 3. Permission conveyed through Copyright Clearance Center, Inc.]*

(continued)

CLASSIC EXPERIMENT *(continued)* **BOX 2-1**

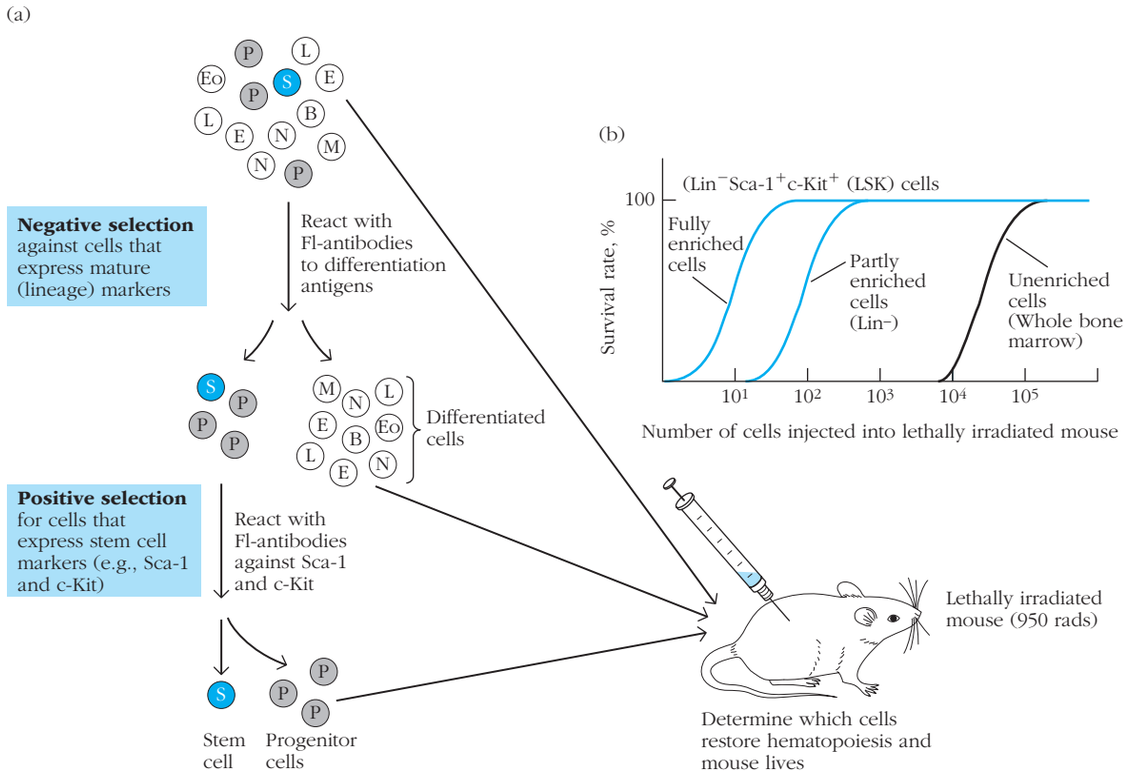


FIGURE 2 **Current approaches for enrichment of pluripotent stem cells from bone marrow.** Shown is a schematic of a commonly used, current approach to enrich stem cells from bone marrow, originated by Irv Weissman and colleagues. (a) Enrichment is accomplished first by *negative selection*: using antibodies to remove cells we don't want. In this case, the undesired cells are the more mature hematopoietic cells (indicated by the white circled letters), which bind to fluorescently labeled antibodies (Fl-antibodies). The next step is *positive selection*: using antibodies to isolate the cells we do want (the stem cells and progenitor cells, indicated by the blue and gray circled letters). In this case the Fl-antibodies are specific for Sca-1 and c-Kit. Abbreviations: S = stem cell; P = progenitor cell; 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 (immunodeficient) mice. Only animals receiving pluripotent stem cells survive. Progressive enrichment for stem cells (from whole bone marrow, to Lin^- cells, to $Lin^-Sca\text{-}1^+c\text{-}Kit^+$ [LSK] cells) is revealed by the decrease in the number of cells needed to restore hematopoiesis. An enrichment of about 1000-fold is possible by this procedure.

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HSCs Differentiate into Myeloid and Lymphoid Blood Cell Lineages

An HSC that is induced to differentiate ultimately loses its ability to self-renew as it progresses from being an LT-HSC to an ST-HSC and then an MPP (**Figure 2-2**). At this stage, a cell makes one of two lineage commitment choices. It can become a **myeloid progenitor cell** (sometimes referred to as a **common myeloid progenitor** or **CMP**), which gives rise to red blood cells, platelets, and myeloid cells (granulocytes, monocytes, macrophages, and some dendritic cell populations). Myeloid cells are members of the innate immune system, and are the first cells to respond to infection or other insults. Alternatively, it can become a **lymphoid progenitor cell** (sometimes referred to as a **common lymphoid progenitor** or **CLP**), which gives rise to B lymphocytes,

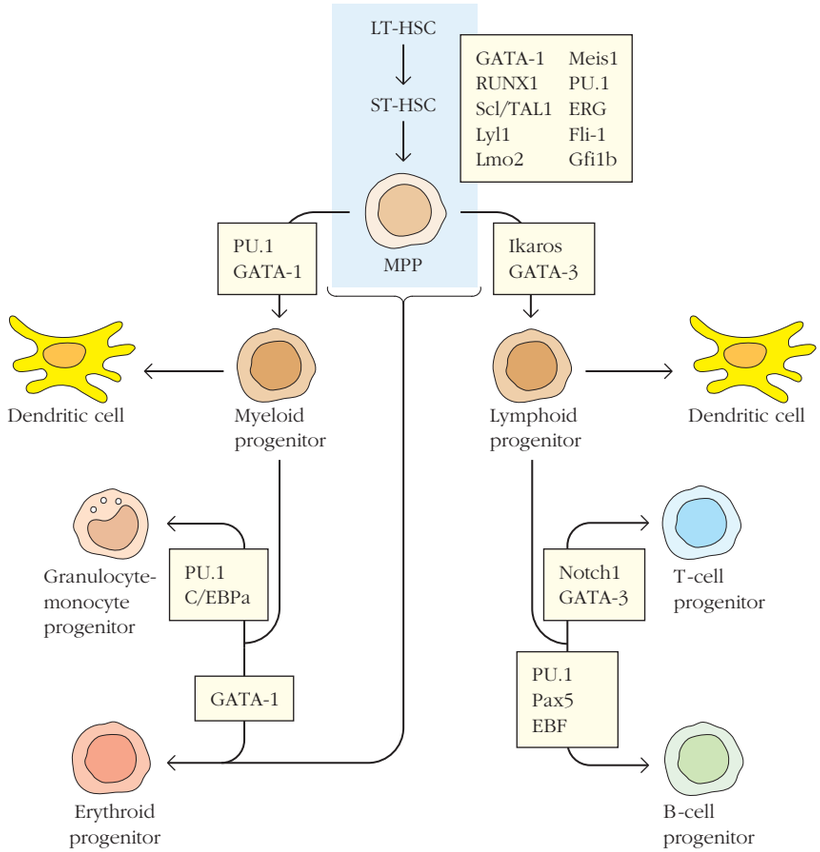


FIGURE 2-2 Regulation of hematopoiesis by transcription factors. A large variety of transcription factors regulate hematopoietic stem cell (HSC) activity (quiescence, self-renewal, multipotency) as well as differentiation to the several lineages that emerge from HSCs. Several key factors are shown here. Note that erythrocytes and megakaryocytes may arise not only from myeloid progenitors, but also from the earliest hematopoietic stem cell populations. Hematopoietic regulation is an active area of investigation; this is one possible schematic based on current information.

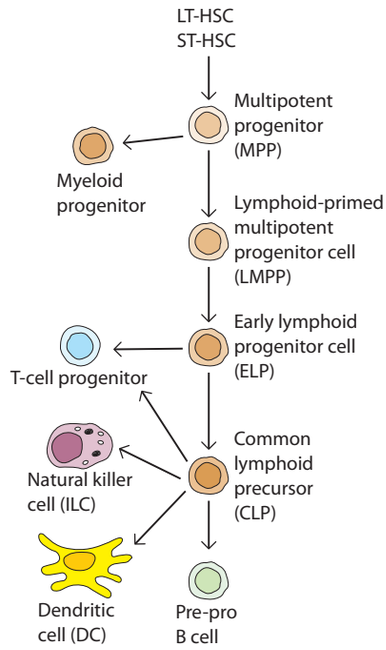
T lymphocytes, innate lymphoid cells (ILCs), as well as specific dendritic cell populations. B and T lymphocytes are members of the adaptive immune response and generate a refined antigen-specific immune response that also gives rise to immune memory. ILCs have features of both innate and adaptive cells.

Recent data suggest that precursors of red blood cells and platelets can arise directly from the earliest LT- and ST-HSC subpopulations (see Figure 2-2). Indeed, the details behind lineage choices are still being worked out by investigators, who continue to identify intermediate cell populations within these broad progenitor categories.

As HSC descendants progress along their chosen lineages, they also progressively lose the capacity to contribute to other cellular lineages. For example, MPPs that are induced to express the receptor Flt-3 lose the ability to become erythrocytes and platelets and are termed ***l*ymphoid-primed, *m*ulti*p*otent *p*rogenitors (LMPPs)** (Figure 2-3). As LMPPs become further committed to the lymphoid lineage, levels of the stem-cell antigens c-Kit and Sca-1 fall, and the cells begin to express RAG1/2 and TdT, enzymes involved in the generation of lymphocyte receptors. Expression of RAG1/2 defines the cell as an ***e*arly *l*ymphoid *p*rogenitor (ELP)**. Some ELPs migrate out of the bone marrow to seed the thymus as T-cell progenitors. The rest of the ELPs remain in the bone marrow as B-cell progenitors. Their levels of the interleukin-7 receptor (IL-7R) increase, and the ELP now develops into a CLP, a progenitor that is now c-Kit^{low}Sca-1^{low}IL-7R^{+} and has lost myeloid potential. However, it still has the potential to mature into any of the lymphocyte lineages: T cell, B cell, or ILC.

Genetic Regulation of Lineage Commitment during Hematopoiesis

Each step a hematopoietic stem cell takes toward commitment to a particular blood cell lineage is accompanied by genetic changes. HSCs maintain a relatively large number of genes in a "primed" state, meaning that they are accessible to transcriptional machinery. Environmental signals that induce HSC differentiation upregulate distinct sets of transcription factors that drive the cell down one of a number of possible developmental pathways. As cells progress down a lineage pathway, primed chromatin regions containing genes that are not needed for the selected developmental



c-Kit	sca-1	flt-3	CD34	IL-7R	Flt-3, Rag1/2, and TdT
+	+	–	–	–	–
+	+	–	+	–	–
+	+	+	–	–	–
+	+	+	–	+/–	+
low	low	+	–	+	+

FIGURE 2-3 An example of lineage commitment during hematopoiesis: the development of B cells from HSCs. The maturation of HSCs into lymphoid progenitors, and the progressive loss of the ability to differentiate into other blood-cell lineages, is exemplified in this figure, which specifically traces the development of B lymphocytes from multipotent progenitors (MPPs). As cells mature from MPP to lymphoid-primed multipotent progenitors (LMPPs) to common lymphoid progenitors (CLPs) they progressively lose the ability to differentiate into other leukocytes. Pre-pro B cells are committed to becoming B lymphocytes. These changes are also accompanied by changes in expression of cell surface markers as well as by the acquisition of RAG and TdT activity.

pathway are shut down. Many transcription factors that regulate hematopoiesis and lineage choices have been identified. Some have distinct functions, but many are involved at several developmental stages and engage in complex regulatory networks. Some transcription factors associated with hematopoiesis are illustrated in Figure 2-2. However, our understanding of their roles continues to evolve.

A suite of factors appear to regulate HSC quiescence, proliferation, and differentiation (see Figure 2-2). Recent sequencing techniques have identified a "top ten" that include GATA-2, RUNX1, Scl/Tal-1, Lyl1, Lmo2, Meis1, PU.1, ERG, Fli-1, and Gfi1b, although others are bound to play a role. Other transcriptional regulators regulate myeloid versus lymphoid cell lineage choices. For instance, *Ikaros* is required for lymphoid but not myeloid development; animals survive in its absence but cannot mount a full immune response (i.e., they are severely *immunocompromised*). Low levels of PU.1 also favor lymphoid differentiation, whereas high levels of PU.1 direct cells to a myeloid fate. Activity of Notch1, one of four Notch family members, induces lymphoid progenitors to develop into T rather than B lymphocytes (see Chapter 8). GATA-1 directs myeloid progenitors toward red blood cell (erythroid) development rather than granulocyte/monocyte lineages. PU.1 also regulates the choice between erythroid and other myeloid cell lineages.

Distinguishing Blood Cells

Historically, investigators classified cells on the basis of their appearance under a microscope, often with the help of dyes. Their observations were especially helpful in distinguishing myeloid from lymphoid lineages, granulocytes from macrophages, and neutrophils from basophils and eosinophils. The pH-sensitive stains hematoxylin and eosin (H&E) are still commonly used in combination to distinguish cell types in blood smears and tissues. The basic dye hematoxylin binds basophilic nucleic acids, staining them blue, and the acidic dye eosin (named for Eos, the goddess of dawn) binds eosinophilic proteins in granules and cytoplasm, staining them pink.

Microscopists drew astute inferences about cell function by detailed examination of stained and unstained cells. Fluorescence microscopy enhanced our ability to identify more molecular details, and in the 1980s, inspired the development of the flow cytometer. This invention revolutionized the study of immunology by allowing us to rapidly measure the presence of multiple surface and internal proteins on individual cells. In vivo cell imaging techniques now permit

TABLE 2-1 Features of cells in human blood

Cell type	Cells/mm³	Total leukocytes (%)	Life span*
Myeloid cells			
Red blood cell	5.0×10^6		120 days
Platelet	2.5×10^5		5–10 days
Neutrophil	$3.7–5.1 \times 10^3$	50–70	6 hours to 2 days
Monocyte	$1–4.4 \times 10^2$	2–12	Days to months
Eosinophil	$1–2.2 \times 10^2$	1–3	5–12 days
Basophil	$<1.3 \times 10^2$	<1	Hours to days
Mast cell	$<1.3 \times 10^2$	<1	Hours to days
Lymphocytes	$1.5–3.0 \times 10^3$	20–40	Days to years
T lymphocytes	$0.54–1.79 \times 10^3$	7–24	
B lymphocytes	$0.07–0.53 \times 10^3$	1–10	
Total leukocytes	7.3×10^3		

*Life spans of cell types in humans are expressed in ranges. Life spans vary, cell populations are heterogeneous (lymphocytes include memory and naïve cells, monocytes circulating in blood could be brand new, or could be coming from tissues, etc.), and measurements depend on experimental conditions.

us to penetrate the complexities of the immune response in time and space. Together with our ever-increasing ability to edit animal and cell genomes, these technologies have revealed an unanticipated diversity of hematopoietic cell types, functions, and interactions. While our understanding of the cell subtypes is impressive, it is by no means complete. **Table 2-1** lists the major myeloid and lymphoid cell types, as well as their life spans and representation in our blood.

Key Concepts:

- HSCs that are induced to differentiate make one of two broad lineage choices. They can give rise to CMPs that develop into myeloid cell types or they can give rise to CLPs that develop into lymphoid cell types. As progenitors differentiate, they progressively lose their ability to self-renew as well as their ability to give rise to other cell lineages.
- Hematopoiesis and lineage choices are regulated by a network of transcription factors including GATA-2, Ikaros, PU.1, and Notch. Environmental signals influence the set of transcription factors expressed by HSCs and thereby determine HSC fate, allowing an organism to develop immune cell subsets according to demand.
- Hematopoietic cells can be distinguished visually using hematoxylin and eosin stains or fluorescent markers. Flow cytometry takes advantage of monoclonal antibodies to distinguish individual cells on the basis of the many surface and internal proteins they express.

Cells of the Myeloid Lineage Are the First Responders to Infection

Myeloid lineage cells include all red blood cells, granulocytes, monocytes, and macrophages. The white blood cells within this lineage are innate immune cells that respond rapidly to the invasion of a pathogen and communicate the presence of an insult to cells of the lymphoid lineage (below). As we will see in Chapter 15, they also contribute to inflammatory diseases (asthma and allergy).

Granulocytes

Granulocytes are often the first responders during an immune response and fall into four main categories: neutrophils, eosinophils, basophils, and mast cells. All granulocytes have multilobed nuclei that make them visually distinctive and easily distinguishable from lymphocytes, whose nuclei are round. Granulocyte subtypes differ by the staining characteristics of their cytoplasmic granules, membrane-bound vesicles that release their contents in response to pathogens (**Figure 2-4**). These granules contain a variety of proteins with distinct functions: some damage pathogens directly; some regulate trafficking and activity of other white blood cells, including lymphocytes; and some contribute to the remodeling of tissues at the site of infection. See **Table 2-2** for a partial list of granule proteins and their functions.

Neutrophils constitute the majority (50% to 70%) of circulating leukocytes (see Figure 2-4a) in adult humans and are much more numerous than eosinophils (1%–3%), basophils (<1%), or mast cells (<1%). After differentiation in

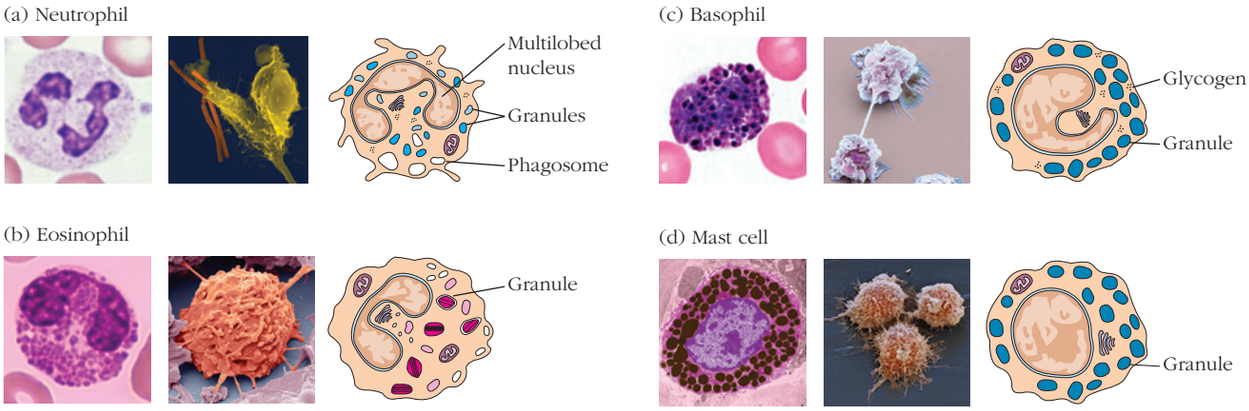


FIGURE 2-4 Examples of granulocytes. (a) Neutrophils, (b) eosinophils, (c) basophils, and (d) mast cells, shown via (*left*) hematoxylin and eosin (H&E) stains of blood smears, (*middle*) scanning electron microscopy (SEM), or (*right*) as a drawing depicting the typical morphology of the indicated granulocyte. Note differences in the shape of the nucleus and in the number, color, and shape of the cytoplasmic granules. *[(a) Neutrophil photos from Science Source/Getty Images (left) and Max Planck Institute for Infection Biology/Dr. Volker Brinkmann. (b) Eosinophil photos from Ed Reschke/Getty Images (left) and Steve Gschmeissner/Science Source (middle). (c) Basophil photos from Michael Ross/Science Source (left) and Steve Gschmeissner/Science Source (middle). (d) Mast cell photos from Biophoto Associates/Science Source (left) and Eye of Science/Science Photo Library (middle).]*

the bone marrow, neutrophils are released into the peripheral blood and circulate for 7 to 10 hours before migrating into the tissues, where they have a life span of only a few days. In response to many types of infection, innate immune cells generate inflammatory molecules (e.g., chemokines) that promote the development of neutrophils in the bone marrow. This transient increase in the number of circulating neutrophils is called **leukocytosis** and is used medically as an indication of infection.

Neutrophils swarm in large numbers to the site of infection in response to inflammatory molecules (**Video 2-4v**). Once in the infected tissue, they phagocytose (engulf) bacteria and secrete a range of proteins that have antimicrobial effects and tissue-remodeling potential. Neutrophils are the main cellular components of pus, where they accumulate at the end of their short lives. Once considered a simple and "disposable" effector cell, neutrophils are now thought to play a regulatory role in shaping the adaptive immune response.

Eosinophils contain granules that stain a brilliant pink in standard H&E staining protocols. They are thought to be important in coordinating our defense against

TABLE 2-2 Examples of proteins contained in neutrophil, eosinophil, and basophil granules

Cell type	Molecule in granule	Examples	Function
Neutrophil	Proteases	Elastase, collagenase	Tissue remodeling
	Antimicrobial proteins	Defensins, lysozyme	Direct harm to pathogens
	Protease inhibitors	α_1-antitrypsin	Regulation of proteases
	Histamine		Vasodilation, inflammation
Eosinophil	Cationic proteins	EPO	Induces formation of ROS
		MBP	Vasodilation, basophil degranulation
	Ribonucleases	ECP, EDN	Antiviral activity
	Cytokines	IL-4, IL-10, IL-13, TNF-α	Modulation of adaptive immune responses
	Chemokines	RANTES, MIP-1α	Attract leukocytes
Basophil/mast cell	Cytokines	IL-4, IL-13	Modulation of adaptive immune
	Lipid mediators	Leukotrienes	Regulation of inflammation
	Histamine		Vasodilation, smooth muscle activation

multicellular parasitic organisms, including helminths (parasitic worms). Eosinophils cluster around invading worms, and damage their membranes by releasing the contents of their eosinophilic granules. Like neutrophils, eosinophils are motile cells (see Figure 2-4b) that migrate from the blood into the tissue spaces. They are most abundant in the small intestines, where their role is still being investigated. In areas where parasites are less of a health problem, eosinophils are better appreciated as contributors to asthma and allergy symptoms. Like neutrophils, eosinophils may also secrete cytokines that regulate B and T lymphocytes, thereby influencing the adaptive immune response.

Basophils are nonphagocytic granulocytes (see Figure 2-4c) that contain large basophilic granules that stain blue in standard H&E staining protocols. Basophils are relatively rare in the circulation, but are potent responders. Like eosinophils, basophils are thought to play a role in our response to parasites, particularly helminths (parasitic worms). When they bind circulating antibody/antigen complexes basophils release the contents of their granules. Histamine, one of the best known compounds in basophilic granules, increases blood vessel permeability and smooth muscle activity, and allows immune cells access to a site of infection. Basophils also release cytokines that can recruit other immune cells, including eosinophils and lymphocytes. In areas where parasitic worm infection is less prevalent, histamines are best appreciated as a cause of allergy symptoms.

Mast cells (see Figure 2-4d) also play a role in combating parasitic worms and contribute to allergies. They are released from the bone marrow into the blood as undifferentiated cells. They mature only after they leave the blood for a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts. Like circulating basophils, these cells have large numbers of cytoplasmic granules that contain histamine and other pharmacologically active substances.

Basophils and mast cells share many features, and basophils were once considered the blood-borne version of mast cells. However, recent data suggest that basophils and mast cells have distinct origins and functions.

Myeloid Antigen-Presenting Cells

Myeloid progenitors also give rise to three groups of phagocytic cells—monocytes, macrophages, and dendritic cells—the cells of each of these groups have **professional antigen-presenting cell (pAPC)** function (**Figure 2-5**).

Professional APCs form important cellular bridges between the innate and adaptive immune systems. They become *activated* after making contact with a pathogen at the site of infection. They communicate this encounter to T lymphocytes in the lymph nodes by displaying peptides from the pathogen to lymphocytes, a process called *antigen presentation* (discussed in Chapter 7). All cells have the capacity to present peptides from internal proteins using MHC class I molecules; however, pAPCs also have the ability to present peptides from external sources using MHC class II molecules (also discussed in Chapter 7). Only MHC class II molecules can be recognized by helper T cells, which initiate the adaptive immune response. (See "Cells of the Lymphoid Lineage" below and Chapter 7 for more information about MHC molecules.)

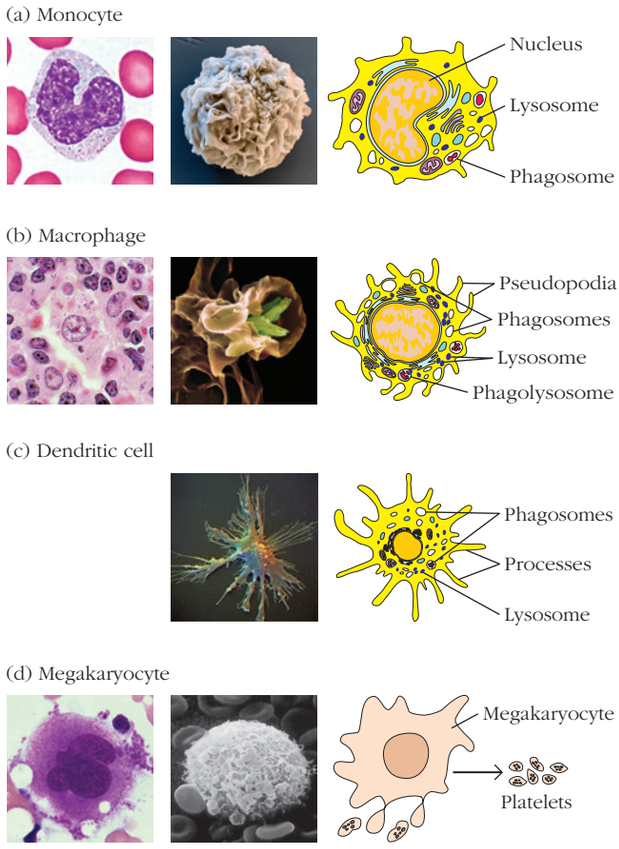


FIGURE 2-5 Examples of monocytes, macrophages, dendritic cells, and megakaryocytes. (a) Monocytes, (b) macrophages, (c) dendritic cells, and (d) megakaryocytes, shown via (*left*) H&E stains of blood smears, (*middle*) SEM, or (*right*) as a drawing depicting the typical morphology of the indicated cell. Note that macrophages are five- to tenfold larger than monocytes and contain more organelles, especially lysosomes. *[(a) Monocyte photos from Michael Ross/Science Source (left) and Eye of Science/Science Source (middle). (b) Macrophage photos from Dr. Thomas Caceci, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia (left) and SPL/Science Source (middle). (c) Dendritic cell photo from David Scharf/Science Source. (d) Megakaryocyte photos from Science Source/Getty Images (left) and Dr. Amar/Science Source (middle).]*

Professional APCs exhibit three major activities when they encounter pathogens (and thereby become activated):

1. They secrete proteins that attract and activate other immune cells.
2. They internalize pathogens via phagocytosis, digest pathogenic proteins into peptides, and then present these peptide antigens on their membrane surfaces via MHC class II molecules.
3. They upregulate *costimulatory molecules* required for optimal activation of helper T cells.

Each variety of pAPC plays a distinct role during the immune response, depending on its locale and its ability to respond to pathogens. Dendritic cells, for example, play a primary role in presenting antigen to—and activating—naïve T lymphocytes (lymphocytes that have not yet been activated by binding antigen). Macrophages are superb phagocytes and are especially efficient at removing both pathogen and damaged host cells from a site of infection. Monocytes regulate inflammatory responses at sites of tissue damage and infection. Investigators have now identified more varieties of APCs than ever anticipated. The functions of these subpopulations are under investigation; some will be described in more detail in coming chapters.

Monocytes constitute from 2% to 12% of white blood cells. They are a heterogeneous group of cells that migrate into tissues and differentiate into a diverse array of tissue-resident phagocytic cells (see Figure 2-5a). Two broad categories of monocytes have been identified. *Inflammatory monocytes* enter tissues quickly in response to infection. *Patrolling monocytes* crawl slowly along blood vessels, monitoring their repair. They also provide a reservoir for tissue-resident monocytes in the absence of infection, and may quell rather than initiate immune responses.

Monocytes that migrate into tissues in response to infection can differentiate into **macrophages** (Figure 2-5b). These inflammatory macrophages are expert phagocytes and typically participate in the innate immune response. They undergo a number of key changes when stimulated by tissue damage or pathogens and have a dual role in the immune response: (1) they contribute directly to the clearance of pathogens from a tissue, and (2) they act as pAPCs for T lymphocytes.

Interestingly, recent work indicates that most tissue-resident macrophages actually arise early in life from embryonic cells rather than from circulating, activated monocytes. These resident macrophages, which include Kupffer cells in the liver, microglia in the brain, and alveolar macrophages in the lungs, have the ability to self-renew and form a committed part of the tissue microenvironment. They co-exist with circulating macrophages and share their function as pAPCs. However, they also assume tissue-specific functions. **Table 2-3** includes a more complete list of tissue-resident macrophages and functions.

Many macrophages express receptors for certain classes of antibody. If a pathogen (e.g., a bacterium) is coated with the appropriate antibody, the complex of antigen and antibody binds to antibody receptors on the macrophage membrane and enhances phagocytosis. In one study, 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, an antibody is an example of an **opsonin**, a molecule that binds an antigen and enhances its recognition

TABLE 2-3 Tissue-specific macrophages

Tissue	Name	Tissue-specific function (in addition to activity as pAPCs)
Brain	Microglia	Neural circuit development (synaptic pruning)
Lung	Alveolar macrophage	Remove pollutants and microbes, clear surfactants
Liver	Kupffer cell	Scavenge red blood cells, clear particles
Kidney	Resident kidney macrophage	Regulate inflammatory responses to antigen filtered from blood
Skin	Langerhans cell	Skin immunity and tolerance
Spleen	Red pulp macrophage	Scavenge red blood cells, recycle iron
Peritoneal cavity	Peritoneal cavity macrophage	Maintain IgA production by B-1 B cells
Intestine	Lamina propria macrophage Intestinal muscularis macrophage	Gut immunity and tolerance Regulate peristalsis
Bone marrow	Bone marrow macrophage	Maintain niche for blood cell development, clear neutrophils
Lymph node	Subcapsular sinus macrophage	Trap antigen particles
Heart	Cardiac macrophage	Clear dying heart cells

Data from Lavin, Y., A. Mortha, A. Rahman, and M. Merad. 2015. Regulation of macrophage development and function in peripheral tissues. *Nature Reviews Immunology* **15:**731; and Mass, E., et al. 2016. Specification of tissue-resident macrophages during organogenesis. *Science* **353:**aaf4238.

and ingestion by phagocytes. The modification of antigens with opsonins is called **opsonization**, a term from the Greek that literally means "to supply food" or "make tasty." Opsonization serves multiple purposes that will be discussed in subsequent chapters.

Ralph Steinman was awarded the Nobel Prize in Physiology or Medicine in 2011 for his discovery of the **dendritic cell (DC)** in the mid-1970s. Dendritic cells (Figure 2-5c) are critical for the initiation of the immune response and acquired their name because they extend and retract long membranous extensions that resemble the dendrites of nerve cells. These processes increase the surface area available for browsing lymphocytes. Dendritic cells are a more diverse population of cells than once was thought, and seem to arise from both the myeloid and lymphoid lineages of hematopoietic cells. The functional distinctions among dendritic cell populations are still being clarified, and each subtype is likely critically important in tailoring immune responses to distinct pathogens and targeting responding cells to distinct tissues.

Dendritic cells perform the distinct functions of antigen capture in one location and antigen presentation in another. Outside lymph nodes, immature forms of these cells monitor the body for signs of invasion by pathogens and capture intruding or foreign antigens. They process these antigens and migrate to lymph nodes, where they present the antigen to naïve T cells, initiating the adaptive immune response.

When acting as sentinels in the periphery, immature dendritic cells take in antigen in three ways. They engulf it by phagocytosis, internalize it by receptor-mediated endocytosis, or imbibe it by pinocytosis. Indeed, immature dendritic cells pinocytose fluid volumes of 1000 to 1500 mm^3 per hour, a volume that rivals that of the cell itself. After antigen contact, they mature from an antigen-capturing phenotype to one that is specialized for presentation of antigen to T cells. In making this transition, some attributes are lost and others are gained. Dendritic cells that have captured antigen lose the capacity for phagocytosis and large-scale pinocytosis. They improve their ability to present antigen and express costimulatory molecules essential for the activation of naïve T cells. After maturation, dendritic cells enter the blood or lymphatic circulation, and migrate to regions containing lymphoid organs, where they present antigen to circulating T cells.

It is important to note that **follicular dendritic cells (FDCs)** do not arise from hematopoietic stem cells and are functionally distinct from dendritic cells. FDCs were named not only for their dendrite-like processes, but for their exclusive location in follicles, organized structures in secondary lymphoid tissue that are rich in B cells. Unlike dendritic cells, FDCs are not pAPCs and do not activate naïve T cells. Instead, they regulate the activation of B cells, as discussed in Chapters 11 and 14.

Erythroid Cells

Cells of the erythroid lineage—**erythrocytes**, or red blood cells—also arise from myeloid progenitors. Erythrocytes contain high concentrations of hemoglobin, and circulate through blood vessels and capillaries delivering oxygen to surrounding cells and tissues. Damaged red blood cells also release signals that induce innate immune activity. In mammals, erythrocytes are anuclear; their nucleated precursors, erythroblasts, extrude their nuclei in the bone marrow. However, the erythrocytes of nonmammalian vertebrates (birds, fish, amphibians, and reptiles) retain their nuclei. Erythrocyte size and shape vary considerably across the animal kingdom—the largest red blood cells can be found among some amphibians, and the smallest among some deer species.

Although the main function of erythrocytes is gas exchange, they may also play a more direct role in immunity. They express surface receptors for antibody and bind antibody complexes that can then be cleared by the many macrophages that scavenge erythrocytes. They also generate compounds, like nitric oxide (NO), that do direct damage to microbes.

Megakaryocytes

Megakaryocytes are large myeloid cells that reside in the bone marrow and give rise to thousands of **platelets**, very small cells (or cell fragments) that circulate in the blood and participate in the formation of blood clots (Figure 2-5d). Clots not only prevent blood loss, but when they take place at epithelial barriers, they also provide a barrier against the invasion of pathogens. Although platelets have some of the properties of independent cells, they do not have their own nuclei.

Key Concepts:

- Granulocytes, including neutrophils, eosinophils, basophils, and mast cells, respond to multiple extracellular pathogens, including bacteria and parasitic worms. When activated, they release the contents of granules, which directly and indirectly impair pathogen activity. These innate immune cells also release cytokines that influence the adaptive immune response and are potent contributors to allergic responses.
- Monocytes, macrophages, and dendritic cells are myeloid cells that, when activated by antigen, are professional antigen-presenting cells (pAPCs) that activate T lymphocytes. Macrophages can be found in all tissues and have two major origins. Some differentiate from circulating monocytes and continue to circulate among tissues. Others, known as tissue-resident macrophages, originate from embryonic cells and do not circulate. They adopt a variety of tissue-specific functions in addition to their role as pAPCs. Dendritic cells are the most potent antigen-presenting cells for naïve T cells.
- Erythrocytes (red blood cells) are anuclear and function primarily in carrying oxygen to cells and tissues. They may also play a direct role in immunity by regulating the clearance of immune complexes and generating antimicrobial compounds.
- Megakaryocytes give rise to platelets, which help generate clots when vessels are damaged.

Cells of the Lymphoid Lineage Regulate the Adaptive Immune Response

Lymphoid lineage cells, or lymphocytes (**Figure 2-6**), are the principal cell players in the adaptive immune response and the source of immune memory. They represent 20% to 40% of circulating white blood cells and 99% of cells in the lymph. Lymphocytes are broadly subdivided into three major populations on the basis of functional and phenotypic differences: B lymphocytes (B cells), T lymphocytes (T cells), and innate lymphoid cells (ILCs), which include the well-understood natural killer (NK) cells. In humans, approximately a trillion (10^{12}) lymphocytes circulate continuously through the blood and lymph and migrate into the tissue spaces and lymphoid organs. Large numbers of lymphocytes reside in the tissues that line our intestines, airways, and reproductive tracts, too. We briefly review the general characteristics and functions of each lymphocyte group and its subsets below.

Small, round, and dominated by their nucleus, lymphocytes are relatively nondescript cells. T and B lymphocytes, in fact, appear identical under a microscope. We therefore rely heavily on the profile of surface proteins they express to differentiate lymphocyte subpopulations.

Surface proteins expressed by cells of the immune system (as well as some other cells) are often referred to by the **cluster of differentiation (CD)** nomenclature. This nomenclature was established in 1982 by an international group of investigators who recognized that many of the new antibodies produced by laboratories all over the world (largely in response to the advent of monoclonal antibody technology) were binding to the same proteins, and hence some proteins were given multiple names by different labs. The group therefore defined clusters of antibodies that appeared to be binding to the same protein and assigned a name—a cluster of differentiation or CD—to each protein. Although originally designed to categorize the multiple antibodies, the CD nomenclature is now firmly associated with specific surface proteins found on cells of many types. **Table 2-4** lists some common CD molecules found on human and mouse lymphocytes. Note that the shift from use of a "common" name to the more standard "CD" name has taken place slowly. For example, investigators often still refer to the pan-T cell marker as "Thy-1" rather than CD90, and the costimulatory molecules as "B7-1" and "B7-2," rather than CD80 and CD86. Appendix I lists over 300 CD markers expressed by immune cells.

B and T cells express many different CD proteins on their surface, depending on their stage of development and state of activation. In addition, each B or T cell also expresses an antigen-specific receptor (the B-cell receptor or the T-cell receptor, respectively) on its surface. Although B and T cell populations express a remarkable diversity of antigen receptors (more than a billion), all antigen-specific receptors on an individual cell's surface are identical in structure and, therefore, are identical in their specificity for antigen. When a particular T or B cell divides, all of its progeny will also

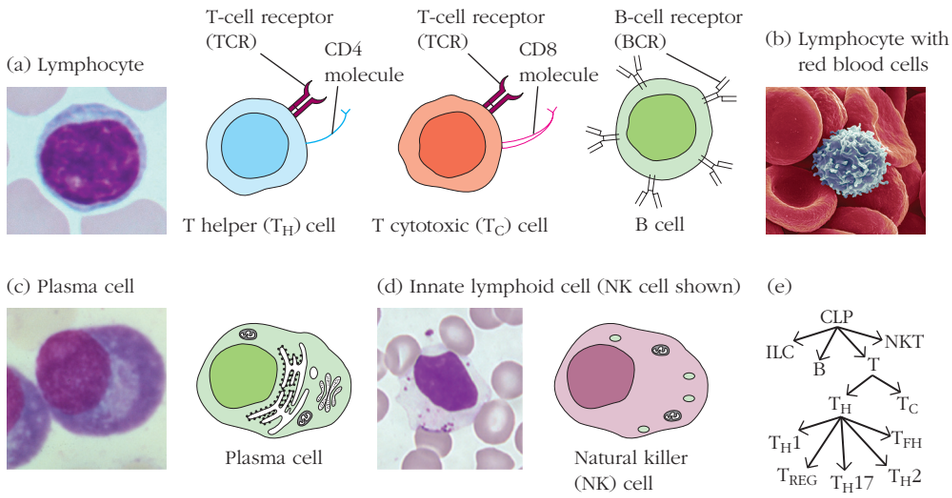


FIGURE 2-6 Examples of lymphocytes. (a) H&E stain of a blood smear showing a typical lymphocyte, with drawings depicting naïve T_H, T_C, and B cells. Note that naïve B cells and T cells look identical by microscopy. (b) SEM of a lymphocyte with red blood cells. (c) H&E stain of a blood smear showing a plasma cell, with a drawing depicting the plasma cell's typical morphology. The cytoplasm of the plasma cell is enlarged because it is occupied by an extensive endoplasmic reticulum network—an indication of the cell's dedication to antibody production. (d) H&E stain of a blood smear showing an innate lymphoid cell, the natural killer (NK) cell. NK cells have more cytoplasm than a naïve lymphocyte; this one is full of granules that are used to kill target cells. (e) A branch diagram that depicts the basic relationship among the lymphocyte subsets described in the text. (Abbreviations: CLP = common lymphoid progenitor; ILC = innate lymphoid cell; T_H1, T_H2, T_H17 = helper T type 1, 2, and 17 cells, respectively; T_{REG} = regulatory T cell.) *[(a) Lymphocyte H&E photo from Science Source/Getty Images. (b) Lymphocyte SEM from Steve Gschmeissner/Science Source. (c) Plasma cell photo © Benjamin Koziner/Phototake. (d) NK photo from Ira Ames, Ph.D., Department of Cell & Developmental Biology, SUNY Upstate Medical University.]*

TABLE 2-4 Common CD markers used to distinguish functional lymphocyte subpopulations

CD designation	Function	B cell	T_H cell	T_C cell	NK cell*
CD2	Adhesion molecule; signal transduction	–	+	+	+
CD3	Signal transduction element of T-cell receptor	–	+	+	–
CD4	Adhesion molecule that binds to MHC class II molecules; signal transduction	–	+ (usually)	– (usually)	–
CD5	Unknown	+ (subset)	+	+	+
CD8	Adhesion molecule that binds to MHC class I molecules; signal transduction	–	– (usually)	+ (usually)	Variable
CD16 (FcγRIII)	Low-affinity receptor for Fc region of IgG	–	–	–	+
CD19	Signal transduction; CD21 coreceptor	+	–	–	–
CD20	Signal transduction; regulates Ca^{2+} transport across the membrane	+	–	–	–
CD21 (CR2)	Receptor for complement (C3d) and Epstein–Barr virus	+	–	–	–
CD28	Receptor for costimulatory 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	–	–	–	+
CD161 (NK1.1)	Lectin-like receptor	–	–	–	+

Synonyms are shown in parentheses.

*NK cells are now considered a cytotoxic member of the innate lymphoid cell (ILC) family. ILCs include three groups of cells that differ by the cytokines they produce. Some classify NK cells within the ILC1 group; others have defined them as a distinct cytotoxic lineage of ILCs.

express this specific antigen receptor. The resulting population of lymphocytes, all arising from the same founding lymphocyte, is a clone (see Figure 1-6).

At any given moment, tens of thousands, perhaps a hundred thousand, distinct mature T- and B-cell clones circulate in a human or mouse, each distinguished by its unique antigen receptor. Newly formed B cells and T cells are considered **naïve**. Contact with antigen induces naïve lymphocytes to proliferate and differentiate into both effector cells and memory cells. **Effector cells** carry out specific functions to combat the pathogen, while **memory cells** persist in the host, and when rechallenged with the same antigen, respond faster and more efficiently. As you have learned in Chapter 1, the first encounter with antigen generates a **primary response**, and the re-encounter a **secondary response** (see Figure 1-8).

B Lymphocytes

The **B lymphocyte (B cell)** derived its letter designation from its site of maturation, in the *b*ursa of Fabricius in birds; the name turned out to be apt, as *b*one marrow is its major site of maturation in humans, mice, and many other mammals. Mature B cells are definitively distinguished from other lymphocytes and all other cells by their expression of the **B-cell receptor** (**BCR**), a membrane-bound immunoglobulin (antibody) molecule that binds to antigen (see **Figure 2-7a** and Chapter 3). Each B cell expresses a surface antibody with a unique specificity, and each of the approximately 1.5–3 $\times$ 10^5 molecules of surface antibody on a B cell has identical binding sites for antigen. B lymphocytes also improve their ability to bind antigen through a process known as *somatic hypermutation* and can generate antibodies of several different functional classes through a process known as *class switching*. Somatic hypermutation and class switching are covered in detail in Chapter 11.

Activated B lymphocytes are the only nonmyeloid cell that can act as a pAPC. They internalize antigen very efficiently via their antigen-specific receptor, and process and present antigenic peptides at the cell surface. Activated B cells also express costimulatory molecules required to activate T cells. By presenting antigen directly to T cells, B cells also receive T-cell help, in the form of cytokines that induce their differentiation into antibody-producing cells (plasma cells) and memory cells.

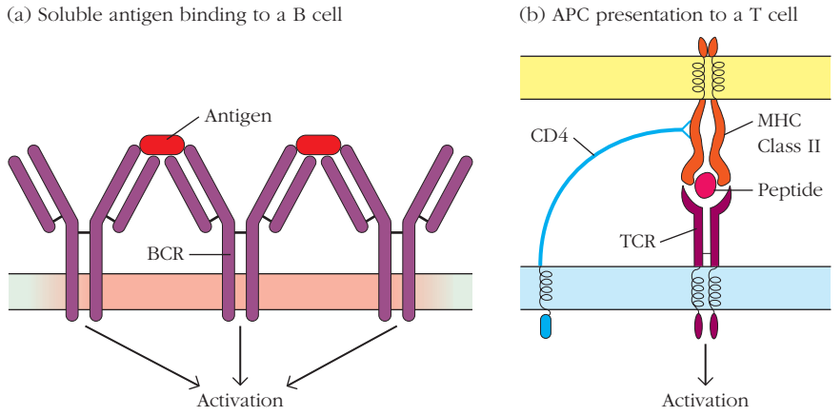


FIGURE 2-7. Structure of the B-cell and T-cell antigen receptors. (a) B-cell receptors recognize soluble antigens. (b) T-cell receptors (TCRs) recognize membrane-bound MHC–peptide complexes. This figure shows a TCR from a helper T cell that recognizes MHC class II (with the assistance of the CD4 molecule). A TCR from a cytotoxic T cell (not shown) would recognize MHC class I with the assistance of a CD8 molecule. (Abbreviations: APC = antigen-presenting cell; BCR = B-cell receptor; MHC = major histocompatibility complex.)

Ultimately, activated B cells differentiate into effector cells known as **plasma cells** (see Figure 2-6c). Plasma cells lose expression of surface immunoglobulin and become highly specialized for secretion of antibody. A single cell is capable of secreting from a few hundred to more than a thousand molecules of antibody per second. Plasma cells do not divide and, although some travel to the bone marrow and live for years, others die within 1 or 2 weeks.

T Lymphocytes

T lymphocytes (T cells) derive their letter designation from their site of maturation in the *t*hymus. Like the B cell, the T cell expresses a unique antigen-binding receptor called the **T-cell receptor** (**TCR**; see Figure 2-7b and Chapter 3). However, unlike membrane-bound antibodies on B cells, which can recognize soluble or particulate antigen, T-cell receptors recognize only processed pieces of antigen (typically peptides) bound to cell membrane proteins called **major histocompatibility complex (MHC) molecules.** MHC molecules are genetically diverse glycoproteins found on cell membranes. They were identified as the cause of rejection of transplanted tissue, and their structure and function are covered in detail in Chapter 7. The ability of MHC molecules to form complexes with antigen allows cells to decorate their surfaces with internal (foreign and self) proteins, exposing them to browsing T cells. MHC comes in two versions: **MHC class I molecules**, which are expressed by nearly all nucleated cells of vertebrate species, and **MHC class II molecules**, which are expressed primarily by pAPCs.

T lymphocytes are divided into two major cell types—**T helper (T_H) cells** and **T cytotoxic (T_C) cells**—that can be distinguished from one another by the presence of either **CD4** or **CD8** membrane glycoproteins on their surfaces. *T cells displaying CD4 generally function as helper (T_H) cells and recognize antigen in complex with MHC class II, whereas those displaying CD8 generally function as cytotoxic (T_C) cells and recognize antigen in complex with MHC class I* (see **Figure 2-8** and Chapter 12). The ratio of $CD4^+$ to $CD8^+$ T cells is approximately 2:1 in healthy mouse and human peripheral blood. A change in this ratio is often an indication of immunodeficiency disease (e.g., HIV infection), autoimmune disease, aging, and inflammation.

Naïve $CD8^+$ T_C cells browse the surfaces of antigen-presenting cells with their T-cell receptors. If and when they bind to an MHC-peptide complex, they become activated, proliferate, and differentiate into a type of effector cell called a **cytotoxic T lymphocyte (CTL)**. The CTL has a vital function in monitoring the cells of the body and eliminating cells that display non-self-antigen complexed with MHC class I, such as virus-infected cells, tumor cells, and cells of a foreign tissue graft. To proliferate and differentiate optimally, naïve $CD8^+$ T cells also need help from mature $CD4^+$ T cells.

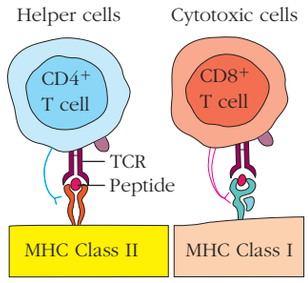


FIGURE 2-8. T-cell recognition of antigen. T cells displaying CD4 generally function as helper (T_H) cells and recognize peptide antigen associated with MHC class II, whereas those displaying CD8 generally function as cytotoxic (T_C) cells and recognize peptide antigen associated with MHC class I.

Naïve CD4$^+$ T_H cells also browse the surfaces of antigen-presenting cells with their T-cell receptors. If and when they recognize an MHC-peptide complex, they become activated and proliferate and differentiate into one of a variety of effector T-cell subsets (see Figure 2-6e). In broad terms, **T helper type 1 (T_H1) cells and T helper type 17 (T_H17) cells** (the latter so named because they secrete IL-17) regulate our response to intracellular pathogens, and **T helper type 2 (T_H2) cells and T follicular helper (T_{FH}) cells** regulate our response to extracellular pathogens, such as bacteria and parasitic worms. **Each CD4$^+$$T_H$-cell subtype** produces a different set of cytokines that enable or "help" the activation of B cells, Tc cells, macrophages, and various other cells that participate in the immune response.

Which helper subtype dominates a response depends largely on what type of pathogen (intracellular versus extracellular, viral, bacterial, fungal, helminth) has infected an animal. The network of cytokines that regulate and are produced by these effector cells is described in detail in Chapter 10.

Another type of CD4$^+$ T cell, the **regulatory T cell (T_{REG})**, has the unique capacity to inhibit immune responses. These cells, called natural T_{REG} cells, arise during maturation in the thymus from cells that bind self proteins with high affinity (autoreactive cells). They also can be *i*nduced at the site of an immune response in an antigen-dependent manner (iT_{REG} cells). Regulatory T cells are identified by the presence of CD4 and CD25 on their surfaces, as well as by the expression of the internal transcription factor FoxP3. T_{REG} cells quell autoreactive responses and play a role in limiting our normal T-cell responses to pathogens.

Both CD4$^+$ and CD8$^+$ T-cell subpopulations may be even more diverse than currently described, and additional functional subtypes could be identified in the future.

NKT Cells

Another type of cell in the lymphoid lineage, the **NKT cell**, shares features with both adaptive and innate immune cells. Like T cells, NKT cells have T-cell receptors (TCRs), and some express CD4. Unlike most T cells, however, the TCRs of NKT cells are not diverse. Rather than recognize protein peptides, they recognize specific lipids and glycolipids presented by a molecule related to MHC proteins known as CD1. NKT cells also have receptors classically associated with innate immune cells, including the NK cells discussed below. Activated NKT cells release cytotoxic granules that kill target cells, but also release large quantities of cytokines that can both enhance and suppress the immune response. They appear to be involved in human asthma, but also may inhibit the development of autoimmunity and cancer. Understanding the exact role of NKT cells in immunity is one research priority.

Innate Lymphoid Cells (ILCs)

Investigators now recognize a group of cells that are derived from common lymphoid progenitors, but do not express antigen-specific receptors: **innate lymphoid cells (ILCs)**. Currently they are subdivided into three groups (ILC1, ILC2, and ILC3), distinguished by the cytokines they secrete, which mirror those produced by distinct helper T-cell subsets (**Table 2-5**). Many provide a first line of defense against pathogens in the skin and at mucosal tissues (Chapter 13). ILC helper subsets are the focus of active investigation, and the nomenclature describing ILC subtypes is still in flux as investigators work to determine their origins and their relationships to each other and to other blood cells.

Cytotoxic natural killer (NK) cells are the founding members of the innate lymphoid cell category and the best studied. Many investigators classify NK cells within the ILC1 group; some define them as a distinct cytotoxic lineage of ILCs. Regardless of their classification, NK cells are

TABLE 2-5 Cytokines secreted by ILC and T-cell subsets

ILC	T-cell subset	Signature cytokines secreted by both	Master transcriptional regulators of both
Group 1 ILCs			
NK cell	CTL	IFN-γ, perforin, granzyme	T-bet
ILC1	T_H1	IFN-γ, TNF	
Group 2 ILCs			
ILC2	T_H2	IL-4, IL-5, IL-13, and amphiregulin	GATA-3
Group 3 ILCs			
LTi cell	T_H17, T_H22	IL-17A, IL-22, LTα, LTβ,	RORγt
ILC3		IL-22, IFN-γ	

Data from Walker, J. A., J. L. Barlow, and A. N. J. McKenzie. 2013. Innate lymphoid cells—how did we miss them? *Nature Reviews Immunology* **13:**75; and Gasteiger, G., and A. Y. Rudensky. 2014. Interactions between innate and adaptive lymphocytes. *Nature Reviews Immunology* **14:**631.

identified by their expression of the NK1.1 surface protein and constitute 5% to 10% of lymphocytes in human peripheral blood.

Efficient cell killers, they use two different strategies to attack a variety of abnormal cells. The first strategy is to attack cells that lack MHC class I molecules. Infection by certain viruses or mutations occurring in tumor cells often cause those cells to downregulate MHC class I. NK cells express a variety of receptors for self-MHC class I that, when engaged, inhibit their ability to kill. However, when NK cells encounter cells that have lost their MHC class I, these inhibiting receptors are no longer engaged and NK cells can release their cytotoxic granules, killing the target cell.

Second, NK cells express receptors (called *Fc receptors* or *FcRs*) for some antibodies. By linking these receptors to antibodies, NK cells can arm themselves with antibodies specific for pathogenic proteins, particularly viral proteins present on the surfaces of infected cells. Once such antibodies bring the NK cell in contact with target cells, the NK cell releases its granules and induces cell death, a process known as *antibody-dependent cell cytotoxicity* (ADCC). The mechanisms of NK-cell cytotoxicity are described further in Chapter 12.

Our understanding of ILCs is still in its infancy, and ongoing investigations are continually generating new insights into their origin and function.

Key Concepts:

- Lymphocytes include B cells, T cells, and innate lymphoid cells (ILCs) and come in many varieties that can be distinguished by patterns of expression of surface CD proteins.
- B and T cells clonally express unique antigen receptors on their surfaces: the B-cell receptor (BCR) and the T-cell receptor (TCR), respectively. Before encountering antigen they are referred to as naïve lymphocytes. After encountering antigen they differentiate into effector and memory lymphocytes.
- The BCR is a membrane version of an antibody. On activation by antigen binding, specificity for the antigen may improve.
- Activated B cells can operate as pAPCs, presenting antigen to T cells; the T cells then directly provide the B cells with the help they need to differentiate.
- B cells ultimately differentiate into antibody-producing cells called plasma cells.
- T lymphocytes express unique antigen receptors (TCRs), and include $CD4^+$ helper T cells ($CD4^+$ T_H), which recognize peptide bound to MHC class II, and $CD8^+$ cytotoxic T cells ($CD8^+$ T_C), which recognize peptide bound to MHC class I.
- Helper T cells come in a variety of subtypes that help tailor our response to distinct pathogens.
- A small population of T cells, called NKT cells, express less diverse TCRs and share features with innate immune cells.
- Innate lymphoid cells (ILCs) include cytotoxic natural killer (NK) cells and several helper cell subsets (ILC1, ILC2, ILC3); these cells do not synthesize antigen-specific receptors but regulate the immune system via the production of cytokines that resemble those generated by helper T cells.
- NK cells have the ability to kill some infected cells and tumor cells.

Primary Lymphoid Organs: Where Immune Cells Develop

HSCs reside in specialized microenvironments, or niches. **Stem cell niches** are sequestered regions lined by supportive cells that regulate stem cell survival, proliferation, differentiation, and trafficking. Microenvironments that nurture HSCs change over the course of embryonic development. By mid to late gestation, HSCs take up residence in the bone marrow, which remains the primary site of hematopoiesis throughout adult life. The bone marrow supports the maturation of all erythroid and other myeloid cells and, in humans and mice, the maturation of B lymphocytes (as described in Chapter 9).

HSCs are also found in blood and may naturally recirculate between the bone marrow and other tissues. This observation has simplified the process used to transplant blood cell progenitors from donors into patients who are deficient (e.g., patients who have undergone chemotherapy). Whereas once it was always necessary to aspirate bone marrow from the donor—a painful process that requires anesthesia—it is now sometimes possible to use enriched hematopoietic precursors from donor blood, which is more easily obtained (see **Clinical Focus Box 2-2**).

Unlike B lymphocytes, T lymphocytes do not complete their maturation in the bone marrow. Instead, T lymphocyte precursors leave the bone marrow and travel to unique microenvironments in the other primary lymphoid organ, the thymus. The structure and function of the thymus will be discussed briefly below and in more detail in Chapter 8.

The Site of Hematopoiesis Changes during Embryonic Development

The bone marrow niche develops late during embryonic development. However, the fetus still needs to generate red and white blood cells required for survival after birth. Where does this happen? During embryogenesis, the site of

CLINICAL FOCUS BOX 2-2

Stem Cells—Clinical Uses and Potential

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

Pluripotent stem cells can give rise to an entire organism. A fertilized egg, the zygote, is an example of such a cell. In humans, the initial divisions of the zygote and its descendants produce cells that are also pluripotent. In fact, identical twins develop when pluripotent cells separate and develop into genetically identical fetuses. *Multipotent stem cells* arise from embryonic stem cells and can give rise to a more limited range of cell types. Further differentiation of multipotent stem cells leads to the formation of *unipotent stem cells*, which can generate only the same cell type as themselves. (Note that "pluripotent" is often used to describe the HSC. Within the context of blood cell lineages this is arguably true; however, it is probably strictly accurate to call the HSC a multipotent stem cell.)

Pluripotent cells, called embryonic stem (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 cells can be induced to generate many different types of cells, including muscle cells, nerve cells, liver cells, pancreatic cells, intestinal epithelial cells, and hematopoietic cells.

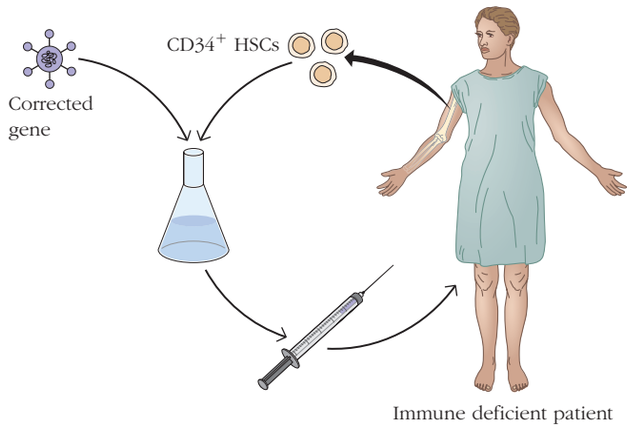


FIGURE 1 **The general strategy used to correct a defective gene by autologous HSC transplantation.** $CD34^+$ HSCs from a patient are removed and infected with a viral vector (e.g., a retrovirus) that carries a corrected version of the gene. The cells with the corrected gene are re-introduced to the patient, who has been treated with chemotherapy or radiation to eliminate the defective blood cells.

Advances have made it possible to grow lines of human pluripotent stem cells and, most recently, to induce differentiated human cells to become pluripotent stem cells. These are developments of considerable importance to the understanding of human development, and they also have great therapeutic potential. In vitro studies of factors that determine or influence the development of human pluripotent stem cells along specific developmental paths are providing considerable insight into how cells differentiate into specialized cell types. This research is driven in part by the great potential for using pluripotent stem cells to generate cells and tissues that could replace diseased or damaged tissue. Success in this endeavor would be a major advance because transplantation medicine now depends entirely on donated organs and tissues, yet the need far exceeds the number of donations, and the need is increasing. Success in deriving cells, tissues, and organs from pluripotent stem cells could 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 the treatment of Parkinson's disease or Alzheimer's disease.

The transplantation of HSCs is an important therapy for patients whose hematopoietic systems must be replaced. It has multiple applications, including:

- Providing a functional immune system to individuals with a genetically determined immunodeficiency, such as severe combined immunodeficiency (SCID).
- Replacing a defective hematopoietic system with a functional one to cure patients with life-threatening nonmalignant genetic disorders in hematopoiesis, such as sickle-cell anemia or thalassemia.
- Restoring the hematopoietic system of cancer patients after treatment with doses of chemotherapeutic agents and radiation. This approach is particularly applicable to

(continued)

CLINICAL FOCUS *(continued)* BOX 2-2

leukemias, including acute myeloid leukemia, which can be cured only by destroying the patient's own hematopoietic system—the source of the leukemia cells. Indeed, any patient receiving therapy based on irradiation or chemotherapeutic regimens that destroy the immune system will benefit from stem cell transplantation.

HSCs have extraordinary powers of regeneration. Experiments in mice indicate that as few as one HSC can completely restore the erythroid population and the immune system. In humans, for instance, as little as 10% of a donor's total volume of bone marrow can provide enough HSCs to completely restore the recipient's hematopoietic system. Once injected into a vein, HSCs enter the circulation and some find their way to the bone marrow, where they begin the process of engraftment. 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 reconstitute their hematopoietic system later, using their own cells.

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 conveyed to the recipient via the graft can attack the recipient's tissues, thereby causing **graft-versus-host disease** (**GVHD**; see Chapter 16), 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 susceptibility to tumors. Consequently, HSC transplantation has the 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, both peripheral blood and umbilical cord blood are now also common sources of HSCs. These alternative sources of HSCs are attractive because the donor does not have to undergo anesthesia or the highly invasive procedure used to extract bone marrow. Although peripheral blood may replace marrow as a major source of HSCs for many applications, bone marrow transplantation still has some advantages (e.g., marrow may include important stem cell subsets that are not as prevalent in blood). 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 *leukapheresis*. If necessary, further purification can be done to remove T cells and to enrich the $CD34^+$ population.

Umbilical cord blood contains an unusually high frequency of HSCs and is obtained from placental tissue that is normally discarded. Consequently, umbilical cord blood has become an attractive source of cells for HSC transplantation. For reasons that are still incompletely understood, however, cord blood stem cell transplants do not engraft as reliably as peripheral blood or bone marrow stem cell transplants.

Beyond its current applications in cancer treatment, autologous stem cell transplantation can also be useful for gene therapy, the introduction of a normal gene to correct a disorder caused by a defective gene. One of the most highly publicized gene therapy efforts—the introduction of the adenosine deaminase (ADA) gene to correct a form of severe combined immunodeficiency (SCID; see Chapter 18)—was performed successfully on HSCs (**Figure 1**). Unfortunately, in a number of patients, the retrovirus used to introduce the corrected ADA gene integrated into parts of the genome that resulted in leukemia. Investigators continue to work to improve the safety and efficiency of gene delivery and have met with more recent successes. New gene editing techniques, including those that involve the CRISPR/Cas9 system (see Chapter 20), may be part of the future for HSC transplantation.

blood cell generation shifts several times before moving into its final home (**Figure 2-9**).

Hematopoiesis begins when precursor cells in the **yolk sac** differentiate into primitive, nucleated erythroid cells that carry the oxygen the embryo needs for early development (7 days after fertilization in the mouse and 3 weeks after fertilization in the human). *Fetal HSCs* capable of generating all blood cell types can be detected close to the developing kidney, specifically in the a*orta*-g*onad*-m*esonephros (AGM) region*, when the fetal heart starts beating. *Mature HSCs* capable of completely repopulating the hematopoietic system of irradiated animals can be isolated from multiple tissues, including the AGM, the *yolk sac*, *placenta*, and *fetal liver*. The placental HSC pool proliferates rapidly and ultimately contains more

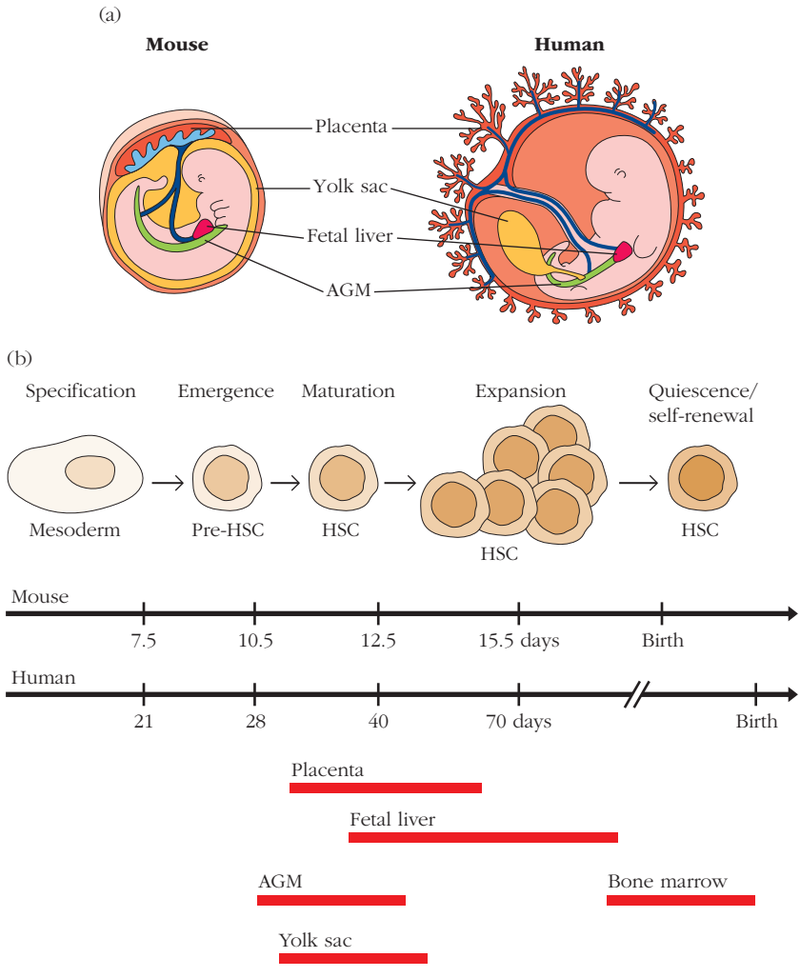


FIGURE 2-9. Sites of hematopoiesis during fetal development. (a) Blood cell precursors are initially found in the yolk sac (yellow) and then spread to the placenta (salmon), fetal liver (pink), and the aorta-gonad-mesonephros (AGM) region (green), before finding their adult home in the bone marrow. The mouse embryo is shown at 11 days of gestation; the human embryo at the equivalent 5 weeks of gestation. (b) The sequence of changes in sites of hematopoiesis during mouse and human embryogenesis. The bars below the time lines indicate when and where functional HSCs are formed.

HSCs than either the AGM or the yolk sac. However, the number of HSCs in the placenta drops as the HSC pool in the fetal liver expands. As an embryo completes its development, the fetal liver is the predominant site of HSC generation.

Within the fetal liver, HSCs form progenitor cells. At the earliest time points, hematopoiesis in the fetal liver is dominated by erythroid progenitors that give rise to the true, enucleated mature erythrocytes that ensure a steady oxygen supply to the growing embryo. Myeloid and lymphoid progenitors emerge gradually. HSCs first seed the bone marrow at late stages in fetal development, and the bone marrow finally takes over as the main site of hematopoiesis, where it will remain throughout postnatal life. Prior to puberty in humans, most of the bones of the skeleton are hematopoietically active, but by the age of 18 years only the vertebrae, ribs, sternum, skull, pelvis, and parts of the humerus and femur retain hematopoietic potential.

The Bone Marrow Is the Main Site of Hematopoiesis in the Adult

The **bone marrow** is the paradigmatic adult stem cell niche (**Figure 2-10**). It is responsible for maintaining the pool of HSCs throughout the life of an adult vertebrate and regulating their differentiation into all blood cell types.

Although the outside surface of a bone is hard, the inside or marrow, also known as the *medullary cavity*, is sponge-like and packed full of cells. A cross-section of a femur (see Figure 2-10b) reveals hematopoietic cells at every stage of differentiation. The medullary cavity can be divided into the **endosteal niche**, lining the bone, and the **perivascular niche**, lining the blood vessels that run through the center of the bone. These niches contain stromal cells that provide structure and guidance for hematopoiesis.

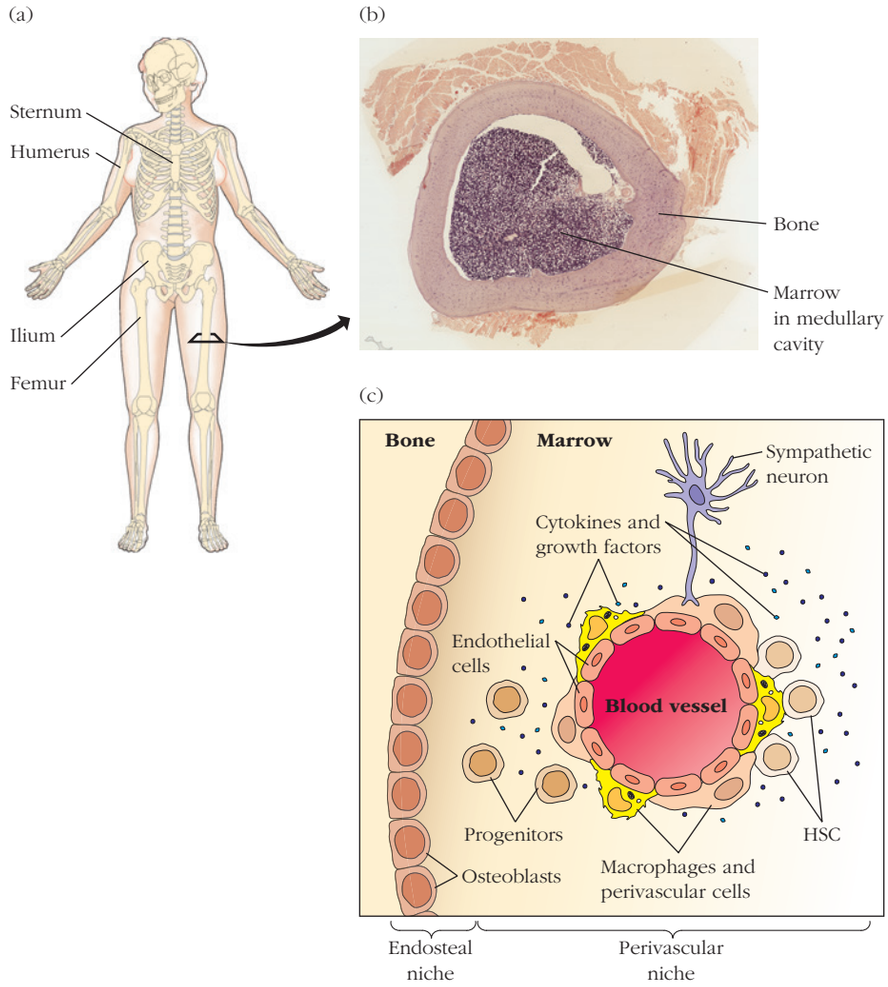


FIGURE 2-10 The bone marrow microenvironment. (a) Multiple bones support hematopoiesis in the adult, including the hip (ilium), femur, sternum, and humerus. (b) This figure shows a cross-section of a bone (femur) with a medullary (marrow) cavity. (c) A schematic of the cross section depicts the perivascular and endosteal niches in more detail. Various cells associated with the central blood vessels generate a niche that supports HSC self-renewal and differentiation. Perivascular cells secrete cytokines and growth factors, and express surface molecules that regulate HSC quiescence and differentiation. Osteoblasts line the bone and provide a niche for developing B cells (not shown). Differentiated cells exit the marrow via blood vessels, which are lined by endothelial cells. *[Photo courtesy of Indiana University School of Medicine, Medical Sciences Program.]*

The stromal cells in the marrow that regulate HSC quiescence, proliferation, trafficking, and differentiation include the following:

1. *Endothelial cells* that line the blood vessels
2. *Perivascular cells* that are diverse in function and interact with endothelial cells
3. *Sympathetic nerves* that transmit signals to other niche cells
4. *Macrophages*, which influence the activity of other niche cells
5. *Osteoblasts*, which generate bone and regulate the differentiation of lymphoid cells

Quiescent, long-lived HSCs are found in the perivascular niche, nurtured by perivascular and endothelial cells (see Figure 2-10c). Some HSCs remain quiescent, while others divide and differentiate into progenitors that develop into myeloid or lymphoid lineages. Specific niches that support myeloid development have not yet been identified. However, the main sites of lymphocyte differentiation are well understood.

B lymphocytes complete most of their development in the bone marrow. Progenitors of B lymphocytes are found in the endosteal niche in association with osteoblasts (Figure 2-10c). More mature B cells are found in the central sinuses of the bone marrow and exit the bone marrow to complete the final stages of their maturation in the spleen. Progenitors of T lymphocytes arise from bone marrow HSCs but exit at a very immature stage and complete their development in the thymus, the primary lymphoid organ for T-cell maturation.

Finally, it is important to recognize that the bone marrow is not only a site for lymphoid and myeloid development but is where fully mature myeloid and lymphoid cells can return. Many mature antibody-secreting B cells (plasma cells) become long-term residents in the bone marrow. Some mature T cells also reside in the bone marrow. Whole bone marrow transplants, therefore, do not simply include stem cells but also include mature, functional cells that can both help and hurt the transplantation effort.

With age, fat cells gradually replace 50% or more of the bone marrow compartment, and the efficiency of hematopoiesis decreases.

Key Concepts:

- HSCs reside primarily in the bone marrow, where stromal cells regulate their quiescence, proliferation, and trafficking. Long-term HSCs reside in the perivascular niche, in association with cells that line the blood vessels.
- In the bone marrow, HSCs differentiate into progenitors, which can become myeloid or lymphoid cell lineages. B lymphocytes complete their maturation in the bone marrow, but progenitors that can differentiate into T lymphocytes exit and complete their maturation in the thymus.

The Thymus Is the Primary Lymphoid Organ Where T Cells Mature

T-cell development is not complete until the cells undergo selection in the **thymus** (**Figure 2-11**). The importance of the thymus in T-cell development was not recognized until the early 1960s, when J. F. A. P. Miller, an Australian biologist, worked against the power of popular assumptions to advance his idea that the thymus was something other than a graveyard for cells. It was an underappreciated organ, very large in prepubescent animals, that was thought by some to be detrimental to an organism, and by others to be an evolutionary dead-end. The cells that populated it—small, thin-rimmed, featureless cells—looked dull and inactive. However, Miller proved that the thymus was the all-important site for the maturation of T lymphocytes (see **Classic Experiment Box 2-3**).

T-cell progenitors, which still retain the ability to give rise to multiple hematopoietic cell types, travel via the blood from the bone marrow to the thymus. The thymus is a specialized environment where immature T cells, known as **thymocytes**, mature into functional T cells by passing through well-defined developmental stages in well-defined microenvironments. Thymocytes ultimately generate unique antigen receptors (T-cell receptors, or TCRs) and are selected to mature on the basis of their TCR reactivity to self-peptide/MHC complexes expressed on the surface of thymic epithelial cells. Thymocytes whose TCRs bind self-MHC/peptide complexes with too-high affinity are induced to die (**negative selection**), and thymocytes that bind self-MHC/peptides with intermediate affinity undergo **positive selection** and mature, migrating to the thymic medulla before entering the circulation. Most thymocytes do not navigate the journey through the thymus successfully; in fact, more than 95% of thymocytes die in transit. The majority of cells die because they have too-low affinity for the self-peptide/MHC combinations that they encounter on the surface of thymic epithelial cells and fail to undergo positive selection, a process called *death by neglect*. T-cell development is discussed in greater detail in Chapter 8.

T-cell development takes place in several distinct thymic microenvironments populated by epithelial cell subtypes (Figure 2-11d). T-cell precursors enter the thymus in blood vessels at the *corticomedullary junction* between the *thymic cortex*, the outer portion of the organ, and the *thymic medulla*, the inner portion of the organ. Thymocytes first travel to the *subcapsular cortex*, just beneath the capsule of the thymus, where they proliferate. They then travel to the cortex, where they first express mature TCRs and interact with cortical thymic epithelial cells (cTECs). Thymocytes that are positively selected in the cortex continue to mature and travel to the medulla, where they interact with medullary thymic epithelial cells (mTECs). Negative selection can happen in any of the microenvironments, although thymocytes are tested for reactivity to tissue-specific antigens in the medulla.

Thymocytes are also distinguished by their expression of two CD antigens, CD4 and CD8 (Figure 2-11d). The most immature thymocytes express neither and are referred to as *double negative* (DN). After entering the cortex, thymocytes upregulate both CD4 and CD8 antigens, becoming *double positive* (DP). As they mature, they lose one or the other CD antigen, becoming *single positive* (SP). $CD4^+$ T cells are helper cells and $CD8^+$ T cells are cytotoxic (killer) cells. Mature SP cells exit the thymus as they entered: via the blood vessels of the corticomedullary

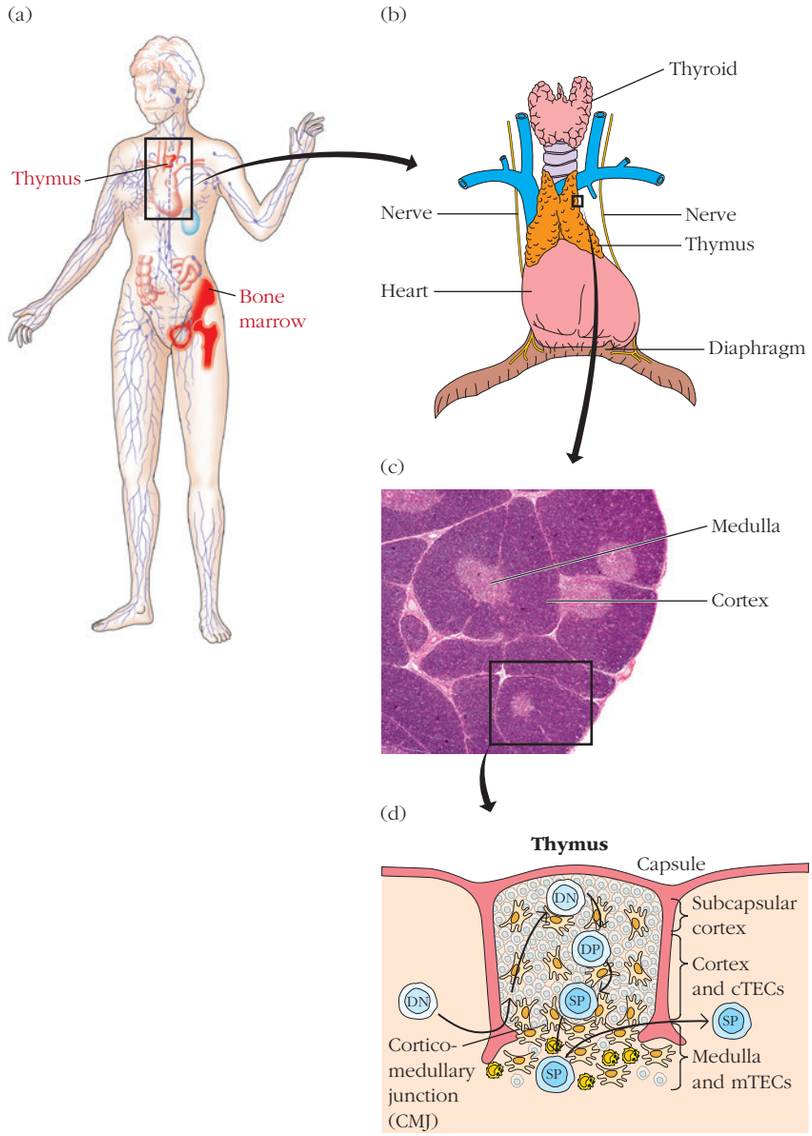


FIGURE 2-11 Structure of the thymus. The thymus is found just above the heart (a and b) and is largest prior to puberty, when it begins to shrink. (c) A stained thymus tissue section, showing the cortex and medulla. (d) A drawing of the microenvironments: the cortex, which is densely populated with double-positive (DP) immature thymocytes (blue cells) and the medulla, which is sparsely populated with single-positive (SP), mature thymocytes. These major regions are separated by the corticomedullary junction (CMJ), where cells enter from and exit to the bloodstream. The area between the cortex and the thymic capsule, the subcapsular cortex, is a site of much proliferation of the youngest, double-negative (DN) thymocytes. The route taken by a typical thymocyte during its development from the DN to DP to SP stages is shown. Thymocytes are positively selected in the cortex. Autoreactive thymocytes are negatively selected in the medulla; some may also be negatively selected in the cortex. (Abbreviations: cTECs = cortical thymic epithelial cells; mTECs = medullary thymic epithelial cells.) *[Photo (c) from Jose Luis Calvo/Shutterstock.]*

junction. Maturation is finalized in the periphery, where these new T cells (*recent thymic emigrants*) explore antigens presented in secondary lymphoid tissue, including the spleen and lymph nodes. T-cell development and positive and negative selection are discussed in more detail in Chapter 8.

CLASSIC EXPERIMENT BOX 2-3

The Discovery of a Thymus—and Two

J. F. A. P. Miller Discovered The Function of The Thymus

In 1961, Miller, who had been investigating the thymus's role in leukemia, published a set of observations in the *Lancet* that challenged notions that, at best, this organ served as a cemetery for lymphocytes and, at worst, was detrimental to health (**Figure 1**). He noted that when this organ was removed in very young mice (in a process known as *thymectomy*), the subjects became susceptible to a variety of infections, failed to reject skin grafts, and died prematurely. On close examination of their circulating blood cells, they also appeared to be missing a type of cell that another investigator, James Gowans, had associated with cellular and humoral immune responses. Miller concluded that the thymus produced functional immune cells.

Several influential investigators could not reproduce the data and questioned Miller's conclusions. Some speculated that the mouse strain he used was peculiar, others that his mice were exposed to too many pathogens and their troubles were secondary to infection. Miller responded to each of these criticisms experimentally, assessing the impact of thymectomy in different mouse strains and in germ-free facilities. His results were unequivocal, and his contention that this organ generated functional lymphocytes was vindicated. Elegant experiments by Miller, James Gowans, and others subsequently showed that the thymus produced a different type of lymphocyte than the bone marrow. This cell did not produce antibodies directly, but, instead, was required for optimal antibody production. It was called a *T cell* after the *t*hymus, its organ of origin. Immature T cells are known as *thymocytes*. Miller is one of the few scientists credited with the discovery of the function of an entire organ.

A Second Thymus

No one expected a new anatomical discovery in immunology in the twenty-first century. However, in 2006 Hans-Reimer Rodewald and colleagues reported the existence of a second thymus in mice. The conventional thymus is a bilobed organ that sits in the thorax right above the heart. Rodewald and colleagues discovered thymic tissue that sits in the neck, near the cervical vertebrae, of mice. This cervical thymic tissue is smaller in mass than the conventional thymus, consists of a single lobe or clusters of single lobes, and is populated by relatively more mature thymocytes. However, it contributes to T-cell development very effectively and clearly contributes to the mature T-cell repertoire. Rodewald's findings raise the possibility that some of our older observations and assumptions about thymic function need to be re-examined. In particular, studies based on thymectomy that indicated T cells could develop outside the thymus may need to be reassessed. The cells found may have come from this more obscure but functional thymic tissue. The evolutionary implications of this thymus are also interesting—thymi are found in the neck in several species, including the koala and kangaroo.

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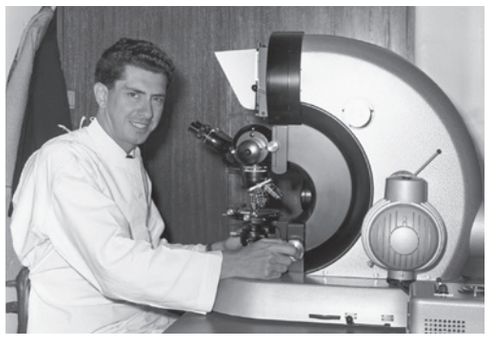

FIGURE 1 **(a) J. F. A. P. Miller in 1961 and (b) the first page of the *Lancet* article (1961) describing his discovery of the function of the thymus.**
[(a) © The Walter and Eliza Hall Institute of Medical Research. (b) Republished with permission of Elsevier, from J.F.A.P. Miller, "Immunological function of the thymus," from The Lancet, 1961 Sept.; 2(7205): 748-749. Permission conveyed through Copyright Clearance Center, Inc.]

(continued)

CLASSIC EXPERIMENT *(continued)* BOX 2-3

(b)

Preliminary Communications

IMMUNOLOGICAL FUNCTION OF THE THYMUS

IT has been suggested that the thymus does not participate in immune reactions. This is because antibody formation has not been demonstrated in the normal thymus,[1] and because, even after intense antigenic stimulation plasma cells (the morphological expression of active antibody formation) and germinal centres have not been described in that organ.[2] Furthermore, thymectomy in the adult animal has had little or no significant effect on antibody production.[3]

On the other hand, there are certain clinical and experimental observations in man and other animals which suggest that the thymus may somehow be concerned in the control of immune responses. Thus, in acute infections, when presumably the need for antibody production is great, the thymus undergoes rapid involution; in patients with acquired agammaglobulinæmia the simultaneous occurrence of benign thymomas has been described,[4] and in fœtal or newborn animals, at a time when responsiveness to antigenic stimulation is deficient, the thymus is a very prominent organ.

The apparent contradiction between these two sets of observations may be partly explained by recent work,[5,6] which suggests that the thymus does not respond to circulating antigens because these cannot reach it owing to the existence of a barrier between the normal gland and the blood-stream. If the barrier is broken, for instance by local trauma, the histological reactions of antibody formation take place in the thymus.

In this laboratory, we have been interested in the role of the thymus in leukæmogenesis.[7] During this work it has become increasingly evident that the thymus at an early stage in life plays a very important part in the development of immunological response.

METHODS AND RESULTS

In the preliminary experiments mice of the C3H and Ak strains and of a cross between T_6 and Ak were used. The thymus was removed 1–16 hours after birth. Alternate littermates were used as sham-thymectomised controls—i.e., they underwent the full operative procedure, including excision of part of the sternum, but their thymuses were left intact. Mice in another group had thymectomy at 5 days of age. Wounds were closed with a continuous black silk suture and the baby mice were returned immediately to their mothers. No antibiotics were administered at any time either to the operated mice or to their mothers.

Mortality during and immediately after the operation ranged between 5 and 15% (excluding deaths due either to neglectful mothers or to cannibalism). Mortality in the thymectomised group was, however, higher between the 1st and 3rd month of life and was attributable mostly to common laboratory infections. This suggested that neonatally thymectomised mice were more susceptible to such infections than even sham-thymectomised littermate controls. When thymectomised and control groups were isolated from other experimental mice and kept under nearly pathogen-free conditions, the mortality in the thymectomised group was significantly reduced.

Absolute and differential white-cell counts were performed on tail blood at various intervals after thymectomy. The significant results of these estimations are summarised in fig. 1. In sham-thymectomised animals the lymphocyte/polymorph ratio rose progressively in the first 8 days of life to reach the normal adult ratio of 2·5±0·08. In the animals whose thymus was removed on the 1st day of life the ratio did not increase significantly and was only 1·0±0·10 at 6 weeks of age.

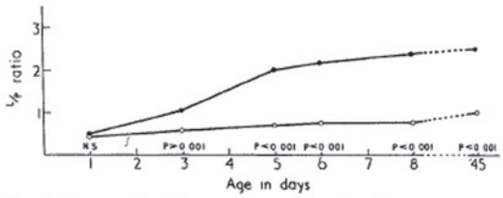

Fig. 1—Average lymphocyte: polymorph ratio of mice thymectomised in the neonatal period compared with sham-thymectomised controls. Statistical differences indicated.
○——○ thymectomised mice.
●——● sham-thymectomised mice.

Histological examination of lymph-nodes and spleens of thymectomised animals at 6 weeks of age revealed a conspicuous deficiency of germinal centres and only few plasma cells (figs. 2 and 3).

At 6 weeks of age, groups of thymectomised, sham-thymectomised, and entirely normal mice were subjected to skin grafting, Ak mice receiving C3H grafts and vice versa, and $(AkXT_6)F_1$ mice receiving C3H grafts. The median survival time of skin grafts in intact mice, sham-thymectomised mice, and mice thymectomised at 5 days of age ranged from 10 to 12 days. In more than 70% of mice whose thymus was removed on the 1st day of life the grafts were established and grew luxuriant crops of hair. Most of these grafts were tolerated for periods ranging from 6 weeks to 2 months and

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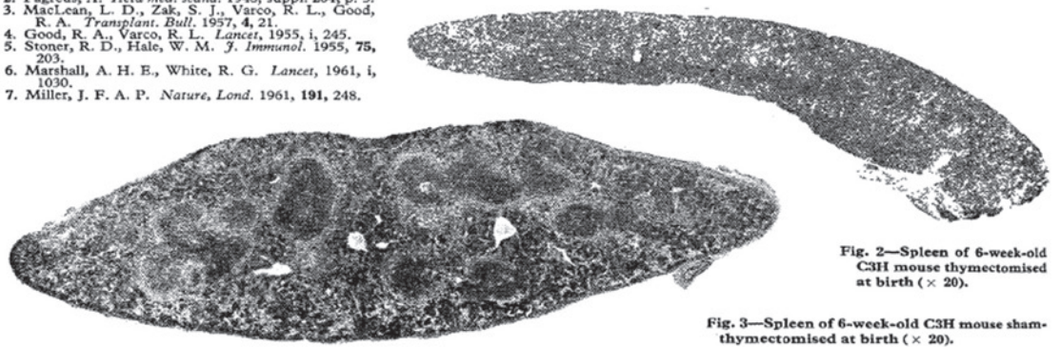

Fig. 2—Spleen of 6-week-old C3H mouse thymectomised at birth (× 20).

Fig. 3—Spleen of 6-week-old C3H mouse sham-thymectomised at birth (× 20).

Key Concepts:

- T-cell progenitors in the bone marrow circulate to the thymus, where they progress through multiple microenvironments and multiple developmental stages and mature into helper $CD4^+$ and cytotoxic $CD8^+$ T lymphocytes.
- Developing T cells (thymocytes) are screened *against* autoreactivity (negative selection) and *for* their ability to recognize self-MHC molecules (positive selection). Only a small percentage survive and reach maturity.

Secondary Lymphoid Organs: Where the Immune Response Is Initiated

As described above, lymphocytes and myeloid cells develop to maturity in the primary lymphoid system: T lymphocytes in the thymus, and B cells, monocytes, dendritic cells, and granulocytes in the bone marrow. However, they encounter antigen and initiate an immune response in the microenvironments of secondary lymphoid organs and tissues.

Secondary Lymphoid Organs Are Distributed throughout the Body and Share Some Anatomical Features

Lymph nodes and the spleen are the most highly organized of the secondary lymphoid organs and are compartmentalized from the rest of the body by a fibrous capsule. Less organized secondary lymphoid tissues are associated with the linings of multiple organ systems, including the skin and the reproductive, respiratory, and gastrointestinal tracts, all of which protect us against external pathogens. These are collectively referred to as **barrier tissues**.

Although secondary lymphoid tissues vary in location and degree of organization, they share key features. All secondary lymphoid structures contain anatomically distinct regions of T-cell and B-cell activity. They also generate lymphoid follicles, highly organized microenvironments responsible for the development and selection of B cells that produce high-affinity antibodies.

Key Concept:

- The immune response to antigen is initiated and organized in secondary lymphoid organs, which include the highly organized lymph nodes and spleen, as well as more loosely organized sites distributed throughout our barrier tissues, including the skin and mucosal membranes.

Blood and Lymphatics Connect Lymphoid Organs and Infected Tissue

Immune cells are highly mobile and use two different systems to traffic through tissues: the blood and lymphatic systems (**Figure 2-12**). Blood vessels have access to virtually every organ and tissue and are lined by endothelial cells that are very responsive to inflammatory signals. Both red and white blood cells transit through the blood—flowing away from the heart via active pumping networks (arteries) and back to the heart via passive valve-based systems (veins)—within minutes. Arteries have thick muscular walls and depend on the beating of the heart to propel cells through vessels. Veins have thinner walls and rely on a combination of internal valves and the activity of muscles to return cells to the heart.

Endothelial cells cooperate with innate immune cells to recruit circulating white blood cells to infected tissue. These cells leave the blood by squeezing between endothelial cells and follow chemokine gradients to the site of infection (see Chapter 14).

Only white blood cells have access to the **lymphatic system**, a network of vessels filled with a protein-rich fluid (**lymph**) derived from the fluid component of blood (**plasma**). These vessels serve, or *drain*, many tissues and provide a route for activated immune cells and antigen to travel from sites of infection to secondary lymphoid organs, where they encounter and activate lymphocytes. Most secondary lymphoid tissues are, in fact, situated along the vessels of the lymphatic system. The spleen is an exception and appears to be served primarily by blood vessels.

Lymphatic vessels also return fluid that seeps from blood capillaries back to the circulatory system (see Figure 2-12c). Depending on the size and activity of an adult, seepage can generate 2.9 liters or more during a 24-hour period. This **interstitial fluid** permeates all tissues and bathes all cells. If this fluid were not returned to the circulation, tissues would swell, resulting in edema (specifically called *lymphedema*) that could become life-threatening. Some individuals are genetically predisposed to lymphedema and others experience it as a result of damage to lymphatic vessels by surgery or trauma.

The walls of the primary lymphatic vessels are thinner than those of blood vessels and more porous. They consist of a single layer of loosely apposed endothelial cells and allow fluids and cells to enter the lymphatic network relatively easily. Within these vessels, the fluid, now called *lymph*, flows into a series of progressively larger collecting vessels called **lymphatic vessels**.

All cells and fluid circulating in the lymph are ultimately returned to the blood system. The largest lymphatic vessel in our bodies, the **thoracic duct**, empties into the left subclavian vein. It collects lymph from all the body except the right arm and right side of the head. Lymph from these areas is collected into the *right lymphatic duct*, which drains into the right subclavian vein (Figure 2-12a). By returning fluid lost from the blood, the lymphatic system ensures steady-state levels of fluid within the circulatory system.

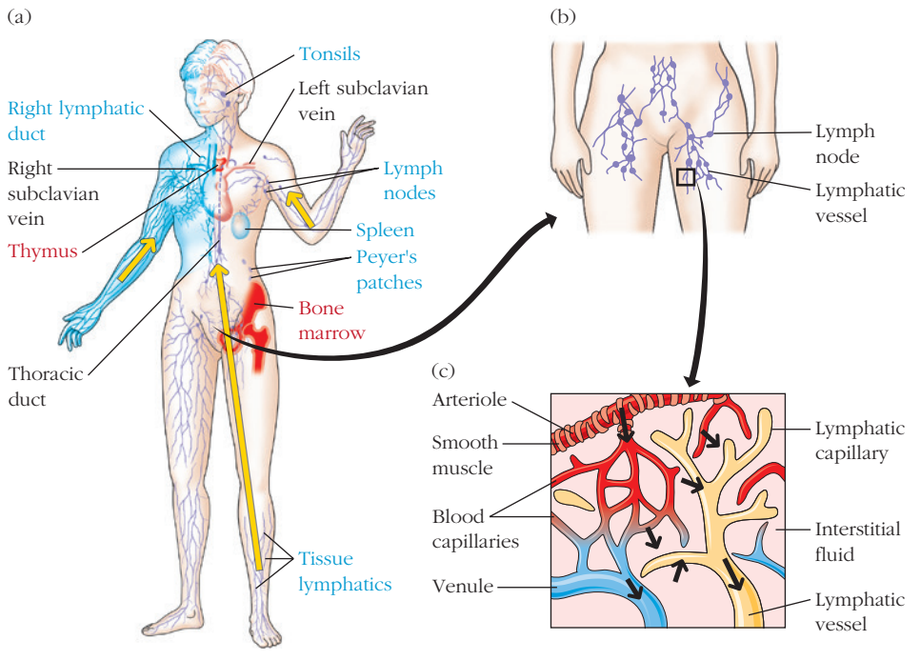

FIGURE 2-12 The human lymphatic system. The primary organs (bone marrow and thymus) are shown in red; secondary organs and tissues, in blue. These structurally and functionally diverse lymphoid organs and tissues are interconnected by the blood vessels (not shown) and lymphatic vessels (purple). Most of the body's lymphatics eventually drain into the thoracic duct, which empties into the left subclavian vein. The vessels draining the right arm and right side of the head (shaded blue) converge to form the right lymphatic duct, which empties into the right subclavian vein. Part (b) shows the lymphatic vessels in more detail, and (c) shows the relationship between blood and lymphatic capillaries in tissue. The lymphatic capillaries pick up interstitial fluid, particulate and soluble proteins, and immune cells from the tissue surrounding the blood capillaries (see arrows).

Like veins, lymphatic vessels rely on a series of one-way valves and the activity of surrounding muscles to establish a slow, low-pressure flow of lymph and cells. Therefore, activity enhances not just venous return, but lymph circulation.

All immune cells that traffic through lymph, blood, and tissues are guided by small molecules known as **chemokines** (see Chapter 3 and Appendix II). Chemokines are chemoattractants secreted by many different cell types including epithelial cells, stromal cells, antigen-presenting cells, lymphocytes, and granulocytes. Chemokine gradients are sensed by immune cells, which express an equally diverse set of chemokine receptors and migrate toward the source of chemokine production.

Key Concepts:

- Both blood and lymphatic vessels carry cells through and between tissues. Red and white blood cells travel from the heart in arteries and return via veins. Some white blood cells and fluid leave the blood to enter tissues. They can be picked up by the lymphatic system, which flows in one direction and ultimately connects back to the bloodstream via the thoracic duct.
- The lymphatic system also transports immune cells and foreign antigens from sites of infection to secondary lymphoid tissues and organs, where the adaptive immune response is activated.

The Lymph Node Is a Highly Specialized Secondary Lymphoid Organ

Lymph nodes (**Figure 2-13**) are the most specialized secondary lymphoid organs. Unlike the spleen, which also regulates red blood cell flow and fate, lymph nodes are fully committed to regulating an immune response. They are encapsulated, bean-shaped structures that include networks of stromal cells (i.e., support tissue) packed with lymphocytes, macrophages, and dendritic cells. Connected to both blood vessels and lymphatic vessels, lymph nodes are the first organized lymphoid structure to encounter antigens that enter the tissue spaces. The lymph node provides ideal microenvironments for encounters between antigen and lymphocytes and productive, organized cellular and humoral immune responses.

Structurally, 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 (see Figure 2-13a). The outermost layer, the **cortex**, contains lymphocytes (mostly B cells), macrophages, and follicular dendritic cells arranged in **follicles**. Beneath the cortex is the **paracortex**, which is populated largely by T lymphocytes but also contains dendritic cells that have migrated into the lymph node from the surrounding tissues (Figure 2-13b and c). The **medulla** is the innermost layer and the site where lymphocytes exit (*egress*) the lymph node through

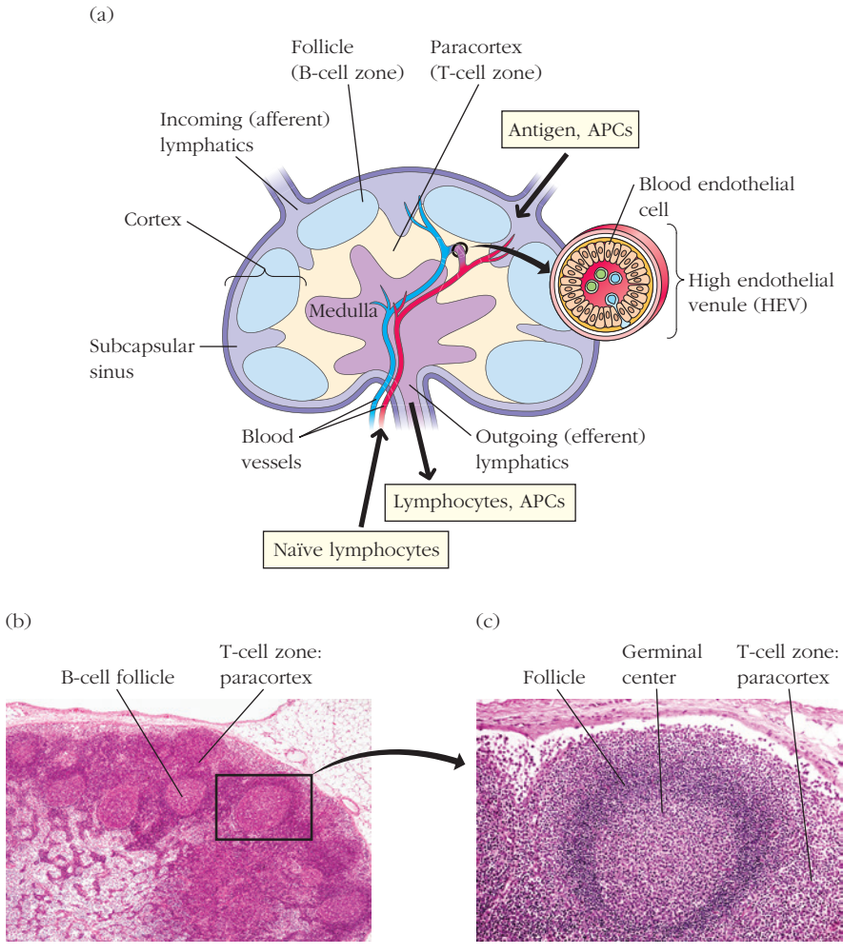

FIGURE 2-13 Structure of a lymph node. The microenvironments of the lymph node support distinct cell activities. (a) A drawing of the major features of a lymph node shows the major vessels that serve the organ: incoming (afferent) and outgoing (efferent) lymphatic vessels, and the arteries and veins. It also depicts the three major tissue layers: the cortex, the paracortex, and the innermost region, the medulla. Macrophages and dendritic cells, which trap antigen, are present in the cortex and paracortex. T cells are concentrated in the paracortex; B cells are primarily in the cortex, within follicles and germinal centers. The medulla is populated largely by antibody-producing plasma cells and is the site where cells exit via the efferent lymphatics. Naïve lymphocytes circulating in the blood enter the lymph node via high endothelial venules (HEVs), in a process called extravasation (see Chapter 14). Antigen and some leukocytes, including antigen-presenting cells (APCs), enter via afferent lymphatic vessels. All cells exit via efferent lymphatic vessels. (b) This stained lymph node section shows the cortex with a number of ovoid follicles, surrounding the T cell–rich paracortex. (c) Another stained lymph node section at higher magnification, showing the T-cell zone and a B-cell follicle that includes a germinal center (also referred to as a *secondary follicle*). *[Photo (b) from Jose Luis Calvo/Shutterstock. Photo (c) from Image Source/Alamy.]*

the outgoing (*efferent*) lymphatics. It is more sparsely populated with lymphoid lineage cells, which include plasma cells that are actively secreting antibody molecules.

Antigen travels from infected tissue to the cortex of the lymph node via the incoming (*afferent*) lymphatic vessels, which pierce the capsule of a lymph node at numerous sites and empty lymph into the *subcapsular sinus* (see Figure 2-13a). It enters either in particulate form or is processed and presented as peptides on the surface of *migrating* antigen-presenting cells. Particulate antigen can be trapped by *resident* antigen-presenting cells in the subcapsular sinus or cortex, where it is passed to other antigen-presenting cells, including B lymphocytes in the follicles. Alternatively, particulate antigen can be processed and presented as peptide-MHC complexes on cell surfaces of resident dendritic cells that are already in the T cell–rich paracortex.

T Cells in the Lymph Node

It takes every naïve T lymphocyte about 16 to 24 hours to browse the MHC-peptide combinations presented by the antigen-presenting cells (APCs) in a single lymph node.

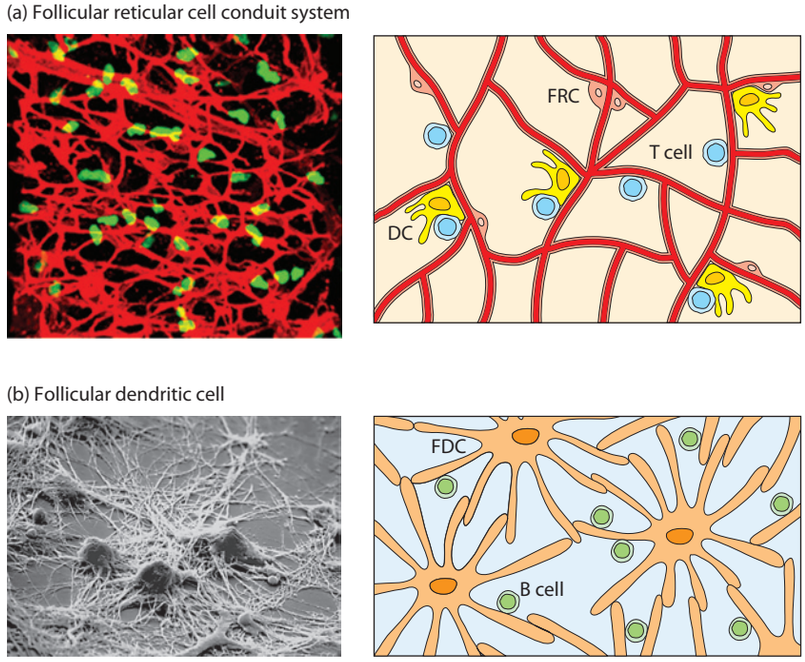

FIGURE 2-14 Stromal cell networks in secondary lymphoid tissue. T and B lymphocytes travel along distinct structures in secondary lymphoid microenvironments. (a) The paracortex is crisscrossed by processes and conduits formed by fibroblastic reticular cells (FRCs), which guide the migration of antigen-presenting cells and T cells, facilitating their interactions. *Left*: An immunofluorescence microscopy image with the FRCs shown in red and the T cells in green. *Right*: A drawing of the network and cell participants. (Abbreviations: DC = dendritic cell; HEV = high endothelial venule.) (b) The B-cell follicle contains a network of follicular dendritic cells (FDCs), which are shown (*left*) as an SEM image as well as (*right*) a drawing. FDCs guide the movements and interactions of B cells. *[Part (a) photo courtesy of Stephanie Favre and Sanjiv A. Luther, University of Lausanne, Switzerland. Part (b) photo courtesy of Mohey Eldin M. El Shikh.]*

Naïve lymphocytes typically enter the cortex of the lymph node via **high endothelial venules** (**HEVs**) of the blood stream. These specialized veins are lined with unusually tall endothelial cells that give them a thickened appearance (Figure 2-13a; and see Figure 14-2). The lymphocytes then squeeze between endothelial cells of the HEV, into the functional tissue of the lymph node.

Once naïve T cells enter the lymph node, they browse MHC–peptide antigen complexes on the surfaces of APCs in the paracortex, the lymph node's **T-cell zone**. The APCs position themselves on a network of fibers that arise from stromal cells called **fibroblastic reticular cells** (**FRCs**) (**Figure 2-14a**). This **fibroblastic reticular cell conduit (FRCC) system** guides T-cell movements via associated adhesion molecules and chemokines. Antigen-presenting cells wrap themselves around the conduits, giving circulating T cells ample opportunity to browse their surfaces as they are guided down the network. The presence of this specialized network elegantly enhances the probability that T cells will meet their specific MHC-peptide combination (see also Chapter 14 opening figure, Figure 14-6, and associated videos 14-Ov and 14-6v).

Although naïve T cells enter via the blood, they exit via the *efferent lymphatics* in the medulla of the lymph node (Figure 2-13a), if they do not find their MHC-peptide match. T cells expressing TCRs that bind an MHC-peptide complex stop migrating and take up residence in the node for several days. Here they proliferate and, depending on cues from the antigen-presenting cell itself, differentiate into effector cells with a variety of distinct functions. $CD8^+$ T cells gain the ability to kill target cells. $CD4^+$ T cells differentiate into several different kinds of effector cells, including those that further activate macrophages, $CD8^+$ T cells, and B cells.

B Cells in the Lymph Node

The lymph node is also the site where B cells are activated and differentiate into high-affinity, antibody-secreting plasma cells. Optimal B-cell activation requires both antigen engagement by the B-cell receptor (BCR) and direct contact with an activated $CD4^+$ T_H cell. Both events are facilitated by the anatomy of the lymph node. Like T cells, B cells circulate through the blood and lymph and visit the lymph nodes on a daily basis, entering via the HEVs. They respond to specific signals and chemokines that draw them not to the paracortex but to the lymph node follicles. Although they may initially take advantage of the FRCC system for guidance, they

ultimately shift to tracts generated by follicular dendritic cells (FDCs) (Figure 2-14b). FDCs maintain follicular and germinal center structure and present particulate antigen to differentiating B cells.

B cells differ from T cells in that their receptors can recognize free, unprocessed antigen. A B cell typically meets its antigen in a lymph node follicle, or **B-cell follicle**. Small, soluble antigens can make their way directly into the follicle, whereas larger antigens are relayed to the follicular dendritic cells by subcapsular macrophages and non-antigen-specific B cells (see Chapter 14). If its BCR binds to antigen, the B cell becomes partially activated and engulfs the antigen and processes it, readying it for presentation as a peptide-MHC complex to $CD4^+$ T_H cells.

B cells that have successfully engaged and processed antigen change their migration patterns and move to the T cell–rich paracortex, where they may encounter a previously activated $CD4^+$ T_H cell. If this helper T cell recognizes the MHC-antigen complex presented by the B cell, the pair will maintain contact for a number of hours, during which the B cell receives signals from the T cell that induce B-cell proliferation and differentiation (see Figure 14-4 and accompanying videos).

Some activated B cells differentiate directly into antibody-producing cells (plasma cells), but others re-enter the follicle to establish a germinal center. A follicle that develops a germinal center is referred to as a **secondary follicle**; a follicle without a germinal center is referred to as a **primary follicle**. In **germinal centers**, B cells proliferate and undergo clonal selection (see Figure 1-6) to produce a colony of B cells with the highest affinity for a particular antigen. Some of these cells travel to the medulla of the lymph node and release antibodies into the bloodstream; others exit through the efferent lymphatics and take up long-term residence in the bone marrow, where they will continue to release antibodies into circulation.

Germinal centers are established within 4 to 7 days of the initial infection, but remain active for 3 weeks or more (Chapter 11). Lymph nodes swell visibly and sometimes painfully during those first few days after infection as immune cells migrate into the node and T and B cells proliferate.

The Generation of Memory T and B Cells in the Lymph Node

Interactions between T cells and APCs, and between activated T_H cells and activated B cells, result in the generation of memory T and B cells. Memory T and B cells either take up residence in secondary lymphoid tissues or exit the lymph node and circulate to and among other tissues, including those that first encountered the pathogen. Memory T cells that reside in secondary lymphoid organs are referred to as *central memory cells* and are distinct in phenotype and functional potential from *effector memory* T cells that circulate among tissues. A third population, *tissue-resident memory* cells, settle in peripheral tissues for the long term and appear to be the first cells to respond when an individual is re-infected with a pathogen. Memory cell phenotype, locale, and activation requirements are very active areas of investigation and will be discussed in more detail in Chapters 10 and 11.

Key Concepts:

- The lymph nodes organize the immune response to antigens that enter through lymphatic vessels. T cells and B cells are compartmentalized in different microenvironments in secondary lymphoid tissue. T cells are found in the paracortex of the lymph nodes, while B cells are organized in follicles in the cortex.
- Naïve T lymphocytes browse the surfaces of antigen-presenting cells in the T-cell zone or paracortex of the lymph node, guided by the FRC network. If they bind to an MHC-peptide combination, they are activated and undergo clonal expansion and differentiation into effector cells in secondary lymphoid organs. If they do not, they exit via the efferent lymphatics and continue browsing in other lymph nodes.
- T lymphocytes develop into mature killer $CD8^+$ and helper $CD4^+$ cells in secondary lymphoid organs. Some $CD4^+$ T cells help B cells to differentiate into antibody-secreting plasma cells; others activate macrophages and $CD8^+$ cytotoxic T cells.
- Naïve B cells that enter lymph nodes travel to B-cell follicles, where they meet their antigen on the FDC network. Those that bind antigen are activated, divide, and seek T-cell help. Those that do not exit the lymph node via the efferent lymphatics to browse another secondary lymphoid tissue.
- Activated B cells undergo further maturation into high-affinity, antibody-producing cells in specialized microenvironments called *germinal centers*, substructures that develop within B-cell follicles.
- B and T cells develop into long-lived memory cells in secondary lymphoid organs. Some memory cells remain in lymphoid tissue, some circulate, and some take up residence in other tissues, ready to respond quickly to a returning pathogen.

The Spleen Organizes the Immune Response against Blood-Borne Pathogens

The **spleen**, situated high in the left side of the abdominal cavity, is a large, ovoid secondary lymphoid organ that plays a major role in mounting immune responses to antigens in the bloodstream (**Figure 2-15**). Whereas lymph nodes are specialized for encounters between lymphocytes and antigen drained from local tissues, the spleen specializes in trapping

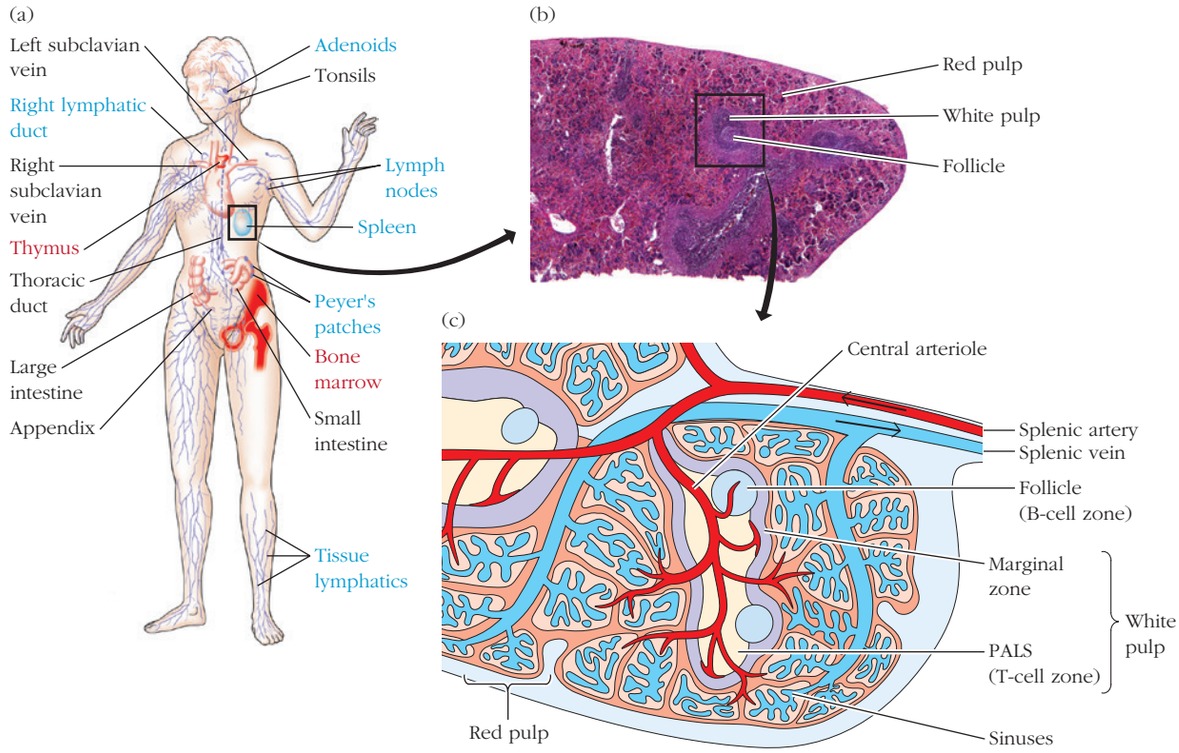

FIGURE 2-15 Structure of the spleen. (a) The spleen, which is about 5 inches long in human adults, is the largest secondary lymphoid organ. It is specialized for trapping blood-borne antigens. (b) A stained tissue section of the human spleen, showing the red pulp, white pulp, and follicles. These microenvironments are diagrammed schematically in (c). 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 is populated by T cells. Closely associated with the PALS are the B cell–rich lymphoid follicles that can develop into secondary follicles containing germinal centers. The marginal zone, a site of specialized macrophages and B cells, surrounds the PALS and separates it from the red pulp. *[Photo (b) courtesy Dr. Keith Wheeler/Science Source.]*

and responding to blood-borne antigens; thus, it is particularly important in the response to systemic infections. Unlike 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** and out via the **splenic vein**. 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 into the interior, dividing the spleen into lobes, all of which function similarly. Two main microenvironmental compartments can be distinguished in each splenic lobe: the **red pulp** and **white pulp**, which are separated by a specialized region called the **marginal zone** (see Figure 2-15c). The splenic red pulp consists of a network of sinusoids populated by red blood cells, macrophages, and some 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-containing pigments from degraded hemoglobin. It is also the site where pathogens first gain access to the lymphoid-rich regions of the spleen, known as the white pulp. The splenic white pulp surrounds the branches of the splenic artery, and consists of B-cell follicles and the **periarteriolar lymphoid sheath** (**PALS**), which is populated by T lymphocytes. As in lymph nodes, germinal centers are generated within these follicles during an immune response. The spleen also maintains a fibroblastic reticular network that provides tracts for T-cell and B-cell migration.

The marginal zone (MZ) is a specialized cellular border between the blood and the white pulp. A relatively recent development in the evolutionary history of the immune system, it is populated by specialized dendritic cells, macrophages, and unique B cells, referred to as *marginal zone B cells* (*MZ B cells*). These cells are the first line of defense

against blood-borne pathogens, trapping antigens that enter via the splenic artery. Marginal zone B cells express both innate immune receptors (e.g., TLRs) and unique B-cell receptors that recognize conserved molecular patterns on pathogens. Once they bind antigen, MZ B cells differentiate rapidly and secrete high levels of antibodies. Although some MZ B cells require T-cell help for activation, others can be stimulated in a T cell–independent manner. Interestingly, mouse and human marginal zone anatomy differ, as does the phenotype and behavior of their marginal zone B cells. The basis for and significance of this species-specific difference are under investigation.

The events that initiate the adaptive immune response in the spleen are analogous to those that occur in the lymph node. Briefly, circulating naïve B cells encounter antigen in the follicles, and circulating naïve $CD8^+$ and $CD4^+$ T cells meet antigen as MHC-peptide complexes on the surface of dendritic cells in the T-cell zone (PALS). Once activated, $CD4^+$ T_H cells then provide help to B cells, including some marginal zone B cells, and $CD8^+$ T cells that have also encountered antigen. Some activated B cells, together with some T_H cells, migrate back into follicles and generate germinal centers. As in the lymph node, germinal center B cells can become memory cells or plasma cells, which circulate to a variety of tissues including the bone marrow.

Children who have undergone **splenectomy** (the surgical removal of a spleen) are vulnerable to *o*verwhelming *p*ost-*s*plenectomy *i*nfection (OPSI) characterized by systemic bacterial infections (sepsis) caused primarily by *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*. Although fewer adverse effects are experienced by adults, splenectomy can still lead to an increased vulnerability to blood-borne bacterial infections, underscoring the role the spleen plays in our immune response to pathogens that enter the circulation. Because the spleen also serves other functions in iron metabolism, platelet storage, and hematopoiesis, these are also compromised if it is removed.

Key Concepts:

- The spleen organizes the first immune response to blood-borne pathogens.
- The spleen is compartmentalized into the white pulp, which contains B and T lymphocytes, and the red pulp, which contains circulating red blood cells.
- The periarteriolar lymphoid sheath of the white pulp includes B-cell follicles and T-cell zones.
- The splenic marginal zone, a specialized region of macrophages and B cells, forms a boundary between the red and the white pulp and plays an important role in trapping and responding to blood-borne antigens.

Barrier Organs Also Have Secondary Lymphoid Tissue

Lymph nodes and the spleen are not the only organs with secondary lymphoid microenvironments. T-cell zones and lymphoid follicles are also found in barrier tissues, which include the skin and mucosal membranes of the digestive, respiratory, and urogenital tracts. Each of these organs is lined by epithelial cells. Our mucosal membranes are lined with a single epithelial layer, while our skin is protected by many layers of epithelial cells. Together, skin and mucosal membranes represent a surface area of over 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 known collectively as **mucosa-associated lymphoid tissue (MALT)**. Lymphoid tissues associated with different mucosal areas are sometimes given more specific names: for instance, **bronchus-associated lymphoid tissue (BALT), nasal-associated lymphoid tissue (NALT)**, **gut-associated lymphoid tissue (GALT), and skin-associated lymphoid tissue (SALT)**.

Each of these tissues plays an important role in our innate immune defenses and recruits many different cell types to the effort. The epithelial cell layers provide more than just physical protection; they also respond actively to pathogens by secreting cytokines, chemokines, and even antimicrobial compounds. Many different types of immune cells reside in the deeper layers of barrier tissues and generate B-cell follicles. B cells that develop in these follicles tend to secrete IgA, which has the ability to cross epithelial barriers and interact with microbes in the lumen of our mucosal tracts.

Innate and adaptive immune cells in barrier organs not only organize our first response to invading pathogens, but they also play a critical role in maintaining tolerance to the diverse and abundant *commensal* microbes that contribute positively to our health. The distinct immune functions and cell residents of each barrier tissue are described in more detail in Chapter 13, but a preview of the organization of secondary lymphoid tissue in the intestine (GALT) is depicted in **Figure 2-16**.

Key Concepts:

- Barrier immune organs, which include the skin and mucosal tissues, contain secondary lymphoid tissue and mount an important first defense against pathogens that penetrate our epithelial layers. Epithelial cells play an active role and initiate the response of innate and adaptive immune cells, which can organize into B-cell follicles.
- Barrier immune systems also help us maintain tolerance to commensal microbes that coexist at our surfaces.

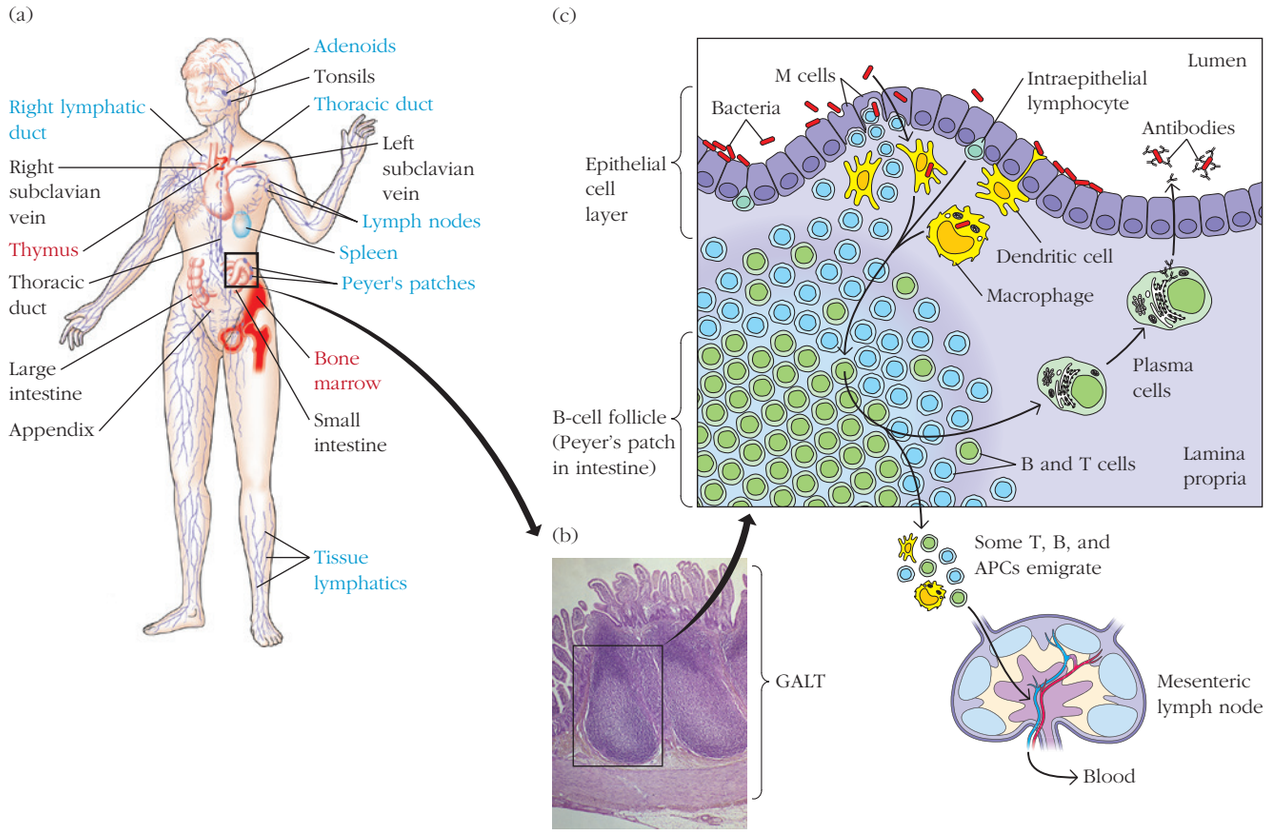

FIGURE 2-16 Example of secondary lymphoid tissue in barrier organs: gut-associated lymphoid tissue (GALT). (a) The Peyer's patch is a representative of the extensive GALT system that is found in the intestine. (b) A stained tissue cross-section of Peyer's patch lymphoid nodules in the intestinal submucosa is schematically diagrammed in (c). The single layer of epithelial cells includes specialized cells, called M cells, that convey antigens from the intestinal lumen to the inner layers (lamina propria) of the intestinal wall. Here they trigger the formation of B-cell follicles, which generate antibody-producing plasma cells. The antibodies pass back into the intestinal lumen and bind to pathogens, protecting the intestinal wall from inflammation and invasion. Other cells, including macrophages, dendritic cells, and intraepithelial lymphocytes, sample antigens from the lumen and, with the help of regulatory T cells, work to distinguish between beneficial commensal bacteria and more dangerous pathogens. Antigen-presenting cells and lymphocytes can travel to local lymph nodes, where they trigger a more systemic immune response to antigens. *[Part (b) photo from Ed Reschke/Getty Images.]*

Tertiary Lymphoid Tissues Also Organize and Maintain an Immune Response

A site of active infection and immune activity is often referred to as a **tertiary lymphoid tissue**. Lymphocytes activated by antigen in secondary lymphoid tissue return to these areas (e.g., lung, liver, brain, skin) as effector cells and can also reside there as tissue-resident memory cells. Tertiary lymphoid tissues can generate new microenvironments that organize lymphocyte responses. The brain, for instance, establishes reticular systems that guide lymphocytes responding to chronic infection with the protozoan that causes toxoplasmosis. Organized aggregations of lymphoid cells are especially prominent at sites of chronic infection and highlight the intimate relationship between immune and nonimmune cells, as well as the plasticity of tissue anatomy. This plasticity is also illustrated by the evolutionary relationships among immune systems and organs (see **Evolution Box 2-4**).

Key Concept:

- Tissues that are sites of infection are referred to as *tertiary tissues*. These sites can also develop organized lymphoid microenvironments, including B-cell follicles.

EVOLUTION BOX 2-4

Variations on Anatomical Themes

All multicellular organisms

defend themselves against pathogens, and all have innate systems of immunity (see Chapter 4). The adaptive immune system appeared about 500 million years ago, with the emergence of vertebrate animals. Only vertebrates generate antigen-specific receptors. Interestingly, however, the location, organization, and function of lymphoid tissues vary widely across the vertebrate subphylum.

Vertebrates range from jawless fishes (Agnatha, the earliest lineages, which are represented by the lamprey eel and hagfish), to cartilaginous fish (e.g., sharks and rays, also called elasmobranchs), which represent the earliest lineages of jawed vertebrates (Gnathostomata), to bony fish, amphibians, reptiles, birds, and mammals. If you view these groups as part of an evolutionary progression, you see that, in general, immune tissues and organs evolved by earlier orders have been retained as newer organs of immunity, such as lymph nodes, have appeared (**Figure 1**). All vertebrates, for example, have gut-associated lymphoid tissue (GALT), but only jawed vertebrates have well-developed thymi and spleens. All vertebrates have two different (B and T) lymphoid cell populations, suggesting that they were present in our common ancestor.

T cells were the first cell population to express a diverse repertoire of antigen receptors, and their appearance is directly and inextricably linked to the appearance of the primary immune organ, the thymus. This dependence is reflected in organisms today: all jawed vertebrates have a thymus, and the thymus is absolutely required for the development of T cells. Recent studies indicate that even jawless vertebrates, including the lamprey, harbor distinct thymic-like (thymoid) tissue in their gill regions (**Figure 2**).

In contrast, B-cell development is not bound to one particular organ. In many adult mammals, including mice and humans, B cells primarily develop in the bone marrow and mature further in the spleen. In cattle and sheep, B-cell maturation shifts from the fetal spleen to a patch of tissue embedded in the wall of the intestine called the ileal Peyer's patch. The rabbit also uses gut-associated tissues, especially the appendix, as primary lymphoid tissue for important steps in the proliferation and diversification of B cells. Recent data suggest that the gut can act as a primary lymphoid organ for generating mature B lymphocytes in most vertebrates, even those that depend largely on the bone marrow.

The sites of B-cell development also vary among nonmammalian vertebrates. In jawless vertebrates, B lymphocyte–like cells develop in distinct regions (called *typhlosoles*) in the kidney and intestine. In sharks, B-cell development shifts from liver to kidney to spleen, and in amphibians and reptiles it shifts from liver and spleen to bone marrow. B-cell development in bony fish takes place primarily within the kidney. In birds, B cells complete their development in a unique lymphoid organ associated with the gut, the bursa of Fabricius (**Figure 3**).

Secondary lymphoid tissues appear to have increased in complexity throughout vertebrate evolution. The spleen is the most ancient secondary lymphoid organ, and lymph nodes are the most recent

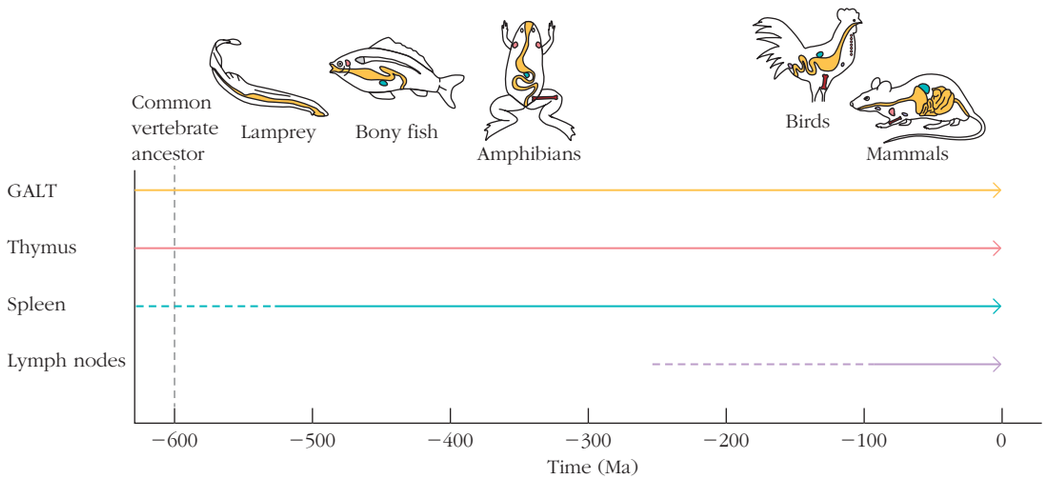

FIGURE 1 **Evolutionary distribution of lymphoid tissues.** The appearance and presence of primary and secondary lymphoid tissues in vertebrates over evolutionary time. (Abbreviations: GALT = **gut-associated lymphoid tissue**; Ma = million years ago.)

(continued)

EVOLUTION *(continued)* **BOX 2-4**

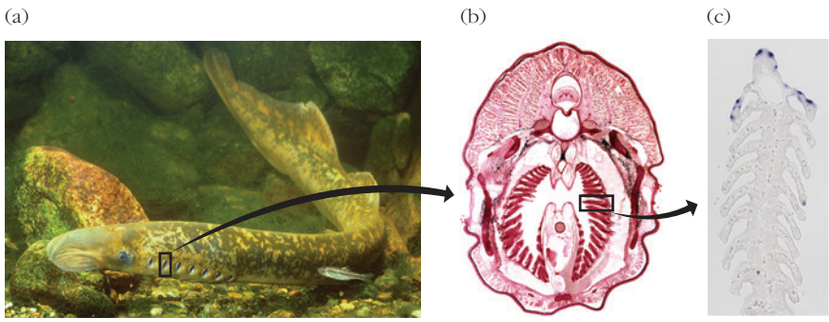

FIGURE 2 **Thymic tissue in the lamprey eel.** (a) Jawless vertebrates, including the lamprey eel, were once thought not to have a thymus. Recent work suggests, however, that they generate two types of lymphocytes analogous to B and T cells and have thymic tissue at the tip of their gills (b). The thymic tissue shown in (c) is stained for expression of CDA1, a gene specific for lymphocytes found in lampreys. *[Left: © Breck P. Kent/Animals Animals-Earth Scenes; all rights reserved. Middle: Dr. Keith Wheeler/Science Source. Right: Courtesy Thomas Boehm.]*

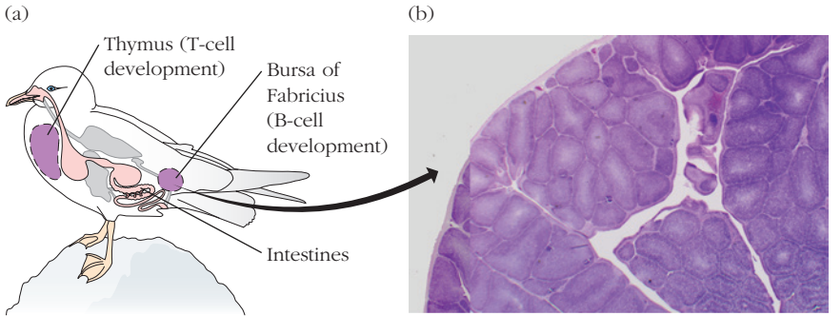

FIGURE 3 **The avian bursa.** (a) Like most vertebrates, birds have a thymus, spleen, and lymph nodes. Although hematopoiesis occurs in their bone marrow, B-cell development takes place in a specialized organ, known as the *bursa*. The bursa is an outpouching of the intestine located close to the cloaca, the common end of the intestinal and genital tracts in birds. A stained tissue section of the bursa and cloaca is shown in (b). *[Photo courtesy Dr. Thomas Caceci, Associate Professor of Biomedical Sciences at the Virginia Tech/Carilion School of Medicine, Roanoke, VA.]*

immune adaptation. Lymph nodes likely arose from tissue associated with lymphatic vessels and appeared first in reptiles and birds. Mammals have the most highly organized lymph nodes, which centralize participants in the adaptive immune response. Secondary lymphoid structures are also found throughout epithelial tissues in vertebrates and vary widely in locale (e.g., although rodents do not have tonsils, they do have well-developed lymphoid tissue at the base of the nose). The general structural and functional features of secondary lymphoid tissue are shared by most vertebrates, with at least one interesting exception: pig lymph nodes exhibit a striking peculiarity—they are "inverted" anatomically, so that the medulla of the organ, where lymphocytes exit the organ, is on the outside, and the cortex, where lymphocytes meet their antigen and proliferate, is on the inside. Lymphocytes exit via blood vessels, rather than via efferent lymphatics. The adaptive advantages, if any, of these odd structural variations are unknown, but the example reminds us of the remarkable plasticity of structure-function relationships within biological structures and the creative opportunism of evolutionary processes.

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Conclusion

All blood cells arise from hematopoietic stem cells, which reside primarily in the adult bone marrow. Immune cells differentiate in primary lymphoid organs, which include the bone marrow and, in the case of T lymphocytes, the thymus. Immune cells differentiate in the bone marrow and thymus (primary lymphoid organs), and then travel through the blood and lymphatics to lymph nodes and the spleen (secondary lymphoid organs), where they browse for antigen. Lymphoid cells circulate to lymph nodes and spleen, secondary lymphoid organs where the adaptive immune response is initiated. Innate immune cells, including APCs and neutrophils, provide the first defense against pathogens that penetrate epithelial barriers. Antigen-presenting cells and antigen travel from the site of infection to the lymph nodes, where they meet and activate browsing T and B lymphocytes. Activated T and B cells differentiate into short-lived effector cells that help clear the infection and long-lived memory cells that protect us against repeat infections.

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Useful Websites

www.bio-alive.com/animations/anatomy.htm A collection of publicly available animations relevant to biology. Scroll through the list to find videos on blood and immune cells, immune responses, and links to interactive sites that reinforce your understanding of immune anatomy.

www.ncbi.nlm.nih.gov/pubmedhealth/PMH0072579/ An accessible site from the U.S. National Library of Medicine that covers the fundamentals of immune system anatomy.

www.niaid.nih.gov/ An accessible site from the National Institute of Allergy and Infectious Diseases about immune system function and structure.

www.hematologyatlas.com/principalpage.htm An interactive atlas of both normal and pathological human blood cells.

https://stemcells.nih.gov Links to information on stem cells, including basic and clinical information, and registries of available embryonic stem cells. The direct link to information on blood stem cells is here:

https://stemcells.nih.gov/info/Regenerative_Medicine/2006Chapter2.htm

www.khanacademy.org/science/health-and-medicine/hematologic-system-diseases-2/leukemia/v/hematopoiesis The Khan Academy's mini-lecture on hematopoiesis.

www.immunity.com/cgi/content/full/21/3/341/DC1 A pair of simulations that trace the activities of T cells, B cells, and dendritic cells in a lymph node. These movies are discussed in more detail in Chapter 14.

www.hhmi.org/research/investigators/cyster.html Investigator Jason Cyster's public website that includes many videos of B-cell activity in immune tissue.

STUDY QUESTIONS

1. Each of the following statements is false. Please correct.

- **a.** Mature T cells are found only in the lymph nodes and spleen.
- **b.** The pluripotent stem cell is one of the most abundant cell types in the bone marrow.
- **c.** There are no stem cells in blood.
- **d.** Activation of macrophages increases their expression of MHC class I molecules, allowing them to present antigen to $CD4^+$ T_H cells more effectively.
- **e.** B cells develop in the thymus.
- **f.** Lymphoid follicles are present only in the spleen and lymph nodes.
- **g.** The FRC guides B cells to follicles.
- **h.** Infection has no influence on the rate of hematopoiesis.
- **i.** Follicular dendritic cells can process and present antigen to T lymphocytes.
- **j.** Dendritic cells arise only from the myeloid lineage.
- **k.** All lymphoid cells have antigen-specific receptors on their membrane.
- **l.** All vertebrates generate B lymphocytes in bone marrow.
- **m.** Only mammals have a thymus.
- **n.** Jawless vertebrates do not have lymphocytes.

2. Identify which of the following cells are myeloid and which are lymphoid.

- **a.** Dendritic cells
- **b.** Neutrophils
- **c.** NK cells
- **d.** Basophils
- **e.** Macrophages
- **f.** T_C cells
- **g.** B cells
- **h.** ILCs

3. List two primary and two secondary lymphoid organs and summarize their functions in the immune response.

4. What two characteristics distinguish HSCs from mature blood cells?

5. How does the thymus help us avoid autoimmune responses?

6. At what age does the thymus reach its maximal size?

- **a.** During the first year of life
- **b.** Teenage years (puberty)
- **c.** Between 40 and 50 years of age
- **d.** After 70 years of age

7. Preparations enriched in HSCs are useful for research and clinical practice. What is the role of immunodeficient mice (i.e., mice that are missing one or more immune cell type) in demonstrating the success of HSC enrichment?

8. Explain the difference between a monocyte and a macrophage.

9. What effect would removal of the bursa of Fabricius (bursectomy) have on chickens?

10. Indicate whether each of the following statements about the lymph node and spleen is true or false. If you think a statement is false, please explain.

- **a.** The lymph node is the first place that immune cells encounter blood-borne antigens.
- **b.** The lymph node paracortex is rich in T cells, and the splenic periarteriolar lymphoid sheath (PALS) is rich in B cells.
- **c.** Only the lymph node contains germinal centers.
- **d.** Fibroblastic reticular cell conduits enhance the probability of T cell–APC interactions.
- **e.** Afferent lymphatic vessels draining the tissue spaces enter the spleen.
- **f.** Lymph node, but not spleen, function is affected by a knockout of the *Ikaros* gene.

11. For each description below (1–15), select the appropriate cell type (a–o). Each cell type may be used once, more than once, or not at all.

DESCRIPTIONS

1. Major cell type presenting antigen to naïve T cells
2. Phagocytic cell of the central nervous system
3. Granulocytic cells important in the body's defense against parasitic organisms
4. Gives rise to red blood cells
5. Generally first cells to arrive at site of inflammation
6. Supports maintenance of HSCs
7. Gives rise to thymocytes
8. Circulating blood cells that differentiate into macrophages in the tissues
9. An antigen-presenting cell that arises from the same precursor as a T cell but not the same as a macrophage
10. Cells that are important in sampling antigens of the intestinal lumen
11. Granulocytic cells that release various pharmacologically active substances
12. White blood cells that play an important role in the development of allergies
13. Cells that can use antibodies to recognize their targets
14. Cells that express antigen-specific receptors
15. Cells that share a common progenitor with T and B cells, but do not have antigen-specific receptors

Cell Types

- **a.** Common myeloid progenitor cells
- **b.** Monocytes
- **c.** Eosinophils
- **d.** Dendritic cells
- **e.** Innate lymphoid cells
- **f.** Mast cells
- **g.** Neutrophils
- **h.** M cells
- **i.** Osteoblasts
- **j.** Lymphocytes
- **k.** NKT cells
- **l.** Microglial cells
- **m.** Myeloid dendritic cells
- **n.** HSCs
- **o.** Lymphoid dendritic cells

RESEARCH FOCUS QUESTION Notch is a surface protein that regulates cell fate. When bound by its ligand, it releases and activates its intracellular region, which regulates new gene transcription. Investigators found that the phenotype of developing cells in the bone marrow differed dramatically when they overexpressed the active, intracellular portion of Notch. In particular, the frequency of BCR^+ cells plummeted, and the frequency of TCR^+ cells increased markedly. Interestingly, other investigators found that when Notch was knocked out, the phenotype of cells in the thymus changed: the frequency of BCR^+ cells increased and the frequency of TCR^+ cells decreased dramatically.

Propose a molecular model to explain these observations, and an experimental approach to begin testing your model.

CLINICAL FOCUS QUESTION T and B cells that differentiate from HSCs recognize as self the bodies in which they differentiate. Suppose a woman donates HSCs to a genetically unrelated man whose hematopoietic system was totally destroyed by a combination of radiation and chemotherapy. Further, suppose that, although most of the donor HSCs differentiate into hematopoietic cells, some differentiate into cells of the pancreas, liver, and heart. Recall from **Clinical Focus Box 2-2** that transplanted lymphocytes can attack the recipient's tissues, causing a graft-versus-host (GVH) reaction. Decide which of the following outcomes is likely and justify your choice.

- **a.** The T cells that arise from the donor HSCs do not attack the pancreatic, heart, and liver cells that arose from donor cells but mount a GVH response against all of the other host cells.
- **b.** The T cells that arise from the donor HSCs mount a GVH response against all of the host cells.
- **c.** The T cells that arise from the donor HSCs attack the pancreatic, heart, and liver cells that arose from donor cells but fail to mount a GVH response against all of the other host cells.
- **d.** The T cells that arise from the donor HSCs do not attack the pancreatic, heart, and liver cells that arose from donor cells and fail to mount a GVH response against all of the other host cells.