Christine Dorresteyn Stevens
Linda E. Miller

Clinical Immunology and Serology A Laboratory Perspective

FOURTH EDITION

Clinical Immunology and Serology

A Laboratory Perspective

Fourth Edition

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A Laboratory Perspective

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To my wonderful family: Eric, Kathy, Hannah, and Matthew, and Kevin, Melissa, Turner, and Avery for their love and encouragement.
— C.D.S.

To my wonderful family, for their love and support; to the Clinical Laboratory Science faculty and the Clinical Immunology laboratory staff at SUNY Upstate Medical University, in appreciation of their expertise and collegiality; and especially to my students, who have inspired me to share my passion for immunology over the years.
— L.E.M.

Preface

The fourth edition of *Clinical Immunology and Serology: A Laboratory Perspective* is built on the success of the first three editions. This text is tailored to meet the needs of clinical laboratory students on both the 2- and 4-year levels. It combines practical information about laboratory testing with a concise discussion of the theory behind the testing. For practicing laboratorians and other allied health professionals, the book may serve as a valuable reference about new developments in the field of immunology.

The organization of the chapters is based on the experience of many years of teaching immunology to clinical laboratory science students. The book is divided into four major sections: I. Nature of the Immune System; II. Basic Immunologic Procedures; III. Immune Disorders; and IV. Serological and Molecular Diagnosis of Infectious Disease. Sections build upon one another, and chapters relate previous material to new material by means of boxes titled Connections and Clinical Correlations. These new features help the students to recall information from previous chapters and to bridge theory with actual clinical diagnosis and testing. Information in the chapters is related to real world events in order to make it more interesting for the student and to show the important role that immunology plays in people's daily lives. New to this edition are the Study Guide Tables at the end of many of the chapters, which can be used as study tools by the students.

All chapters have been updated to include new information about the immune system as well as new treatments for immunologic diseases. With this edition comes added emphasis on the basic immune mechanisms. Three new chapters have been added: Innate Immunity (Chapter 3), Adaptive Immunity (Chapter 4), and Immunization and Vaccines (Chapter 25). These chapters have been added in response to comments from reviewers and readers, as well as the burgeoning information in these areas. The chapter on autoimmunity (Chapter 15) has been expanded to include some diseases that are increasing in importance. Additionally, Molecular Diagnostic Techniques (Chapter 12) and Tumor Immunology (Chapter 17) have been expanded to help bring readers up-to-date on new developments in the field. Information on quality assessment, regulatory issues, and quality management systems has been added to the chapter on laboratory safety. Perhaps the most exciting new change, however, is the addition of full color illustrations. Not only does this increase the visual appeal of the book, but full color is helpful to students in promoting a better understanding of principles and techniques discussed in the chapters.

The book remains a practical introduction to the field of clinical immunology that combines essential theoretical principles with serological techniques commonly used in the clinical laboratory. The theory is comprehensive but concise, and the emphasis is on direct application to the clinical laboratory. The text is readable and user-friendly, with learning outcomes, chapter outlines, and a glossary of all key terms. Each chapter is a complete learning module that contains theoretical principles, illustrations, definitions of relevant terminology, and questions and case studies that help to evaluate learning. For the instructor, there are many new online resources at Davis*Plus* to assist in course development. These resources include PowerPoint slides, suggested laboratory exercises, additional case studies, and a large bank of test questions that can be used for review or test preparation. Because the field of immunology is expanding so rapidly, the challenge in writing this book has been to ensure adequate coverage but to keep it on an introductory level. Every chapter has been revised to include current practices as of the time of writing. It is hoped that this book will kindle an interest in both students and laboratory professionals in this exciting and dynamic field.

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Finally, we would like to acknowledge all the folks at F.A. Davis for their hard work in making this fourth edition a reality. To the Senior Acquisitions Editor for Health Professions, Christa Fratantoro, you were a gentle taskmaster who kept us going, and you were a wonderful listener and problem solver when we needed help. We also appreciate the efforts of Dean DeChambeau, our developmental editor, whose thoroughness and promptness were invaluable in keeping us on track. Thanks to George Lang and all the others behind the scenes who helped this book come to life.

Our immunology students—past, present, and future—are the reason for writing this book. We hope that this text will help make a very complex subject a little easier to understand.

A thank you to friends, especially Bayard, for their encouragement and interest.

Finally, to my two wonderful sons Eric and Kevin, their wives Kathy and Melissa, and my grandchildren, Hannah, Turner, Matthew, and Avery, you are my inspiration and my source of strength.

Contents

SECTION IV

1

Nature of the Immune System

1 Introduction to Immunity and the Immune System

Christine Dorresteyn Stevens, EdD, MT(ASCP)

LEARNING OUTCOMES

After finishing this chapter, you should be able to:

1. Discuss how immunology as a science began with the study of immunity.
2. Describe what is meant by an attenuated vaccine.
3. Explain how the controversy over humoral versus cellular immunity contributed to expanding knowledge in the field of immunology.
4. Distinguish innate from adaptive immunity.
5. Describe the types of white blood cells (WBCs) capable of phagocytosis.
6. Explain the role of tissue cells in immunity.
7. Discuss how natural killer (NK) cells differ from T lymphocytes.
8. Identify the two primary lymphoid organs and discuss the main functions of each.
9. List four secondary lymphoid organs and discuss their overall importance to immunity.
10. Describe the function and architecture of a lymph node.
11. Compare a primary and a secondary follicle.
12. Explain the makeup of a cluster of differentiation.
13. Differentiate the roles of T cells and B cells in the immune response.

CHAPTER OUTLINE

You can go to Davis*Plus* at **davisplus.fadavis.com** keyword Stevens for the laboratory exercises that accompany this text.

KEY TERMS

Adaptive immunity
Antibodies
Antigens
Attenuation
Basophils
Bone marrow
Cell-mediated immunity
Chemotaxins
Clusters of differentiation (CD)
Cytokines
Dendritic cells
Diapedesis
Eosinophils
Germinal center
Humoral immunity
Immunity
Immunology
Innate (natural) immunity
Leukocytes
Lymph nodes
Lymphocyte
Macrophages
Mast cells
Memory cells
Monocytes
Natural killer (NK) cells
Neutrophil
Periarteriolar lymphoid sheath (PALS)
Phagocytosis
Plasma cells
Primary follicles
Primary lymphoid organs
Secondary follicles
Secondary lymphoid organs
Spleen
Thymocytes
Thymus

Although humans have been trying for many centuries to unravel the secrets of preventing disease, the field of immunology is a relatively new science. **Immunology** can be defined as the study of a host's reactions when foreign substances are introduced into the body. Such foreign substances that induce a host response are called **antigens.** Antigens are all around us in nature and they vary from substances such as pollen that may make us sneeze to serious bacterial pathogens such as *Staphylococcus aureus* or *Group A Streptococcus* that can cause life-threatening illnesses. The study of immunology has given us the ability to prevent diseases such as smallpox, polio, diphtheria, and measles through the development of vaccines. In addition, understanding how the immune system works has made successful organ transplantation possible and has given us new tools to treat diseases such as cancer and certain autoimmune diseases. Immunological techniques have affected testing in many areas of the clinical laboratory and allowed for such testing to be more precise and automated. Thus, the study of immunology is important to many areas of medicine. In this chapter, we will provide a brief look at the history of the field and then introduce the cells and tissues of the immune system to form a basis for understanding how the immune system works. In later chapters we will apply this knowledge to principles of testing for specific diseases.

Immunity and Immunization

Immunology as a science has its roots in the study of **immunity:** the condition of being resistant to infection. The first recorded attempts to deliberately induce immunity date back to the 1500s when the Chinese inhaled powder made from smallpox scabs in order to produce protection against this dreaded disease. The hypothesis was that if a healthy individual was exposed as a child or young adult the effects of the disease would be minimized. However, the early exposure did not always work. Further refinements did not occur until the late 1700s when an English country doctor by the name of Edward Jenner was able to successfully prevent infection with smallpox by injecting a more harmless substance—cowpox—from a disease affecting cows.[1] Details of the development of this first vaccine can be found in Chapter 25.

The next major development in disease prevention did not occur until almost a hundred years later when Louis Pasteur, often called the father of immunology, observed by chance that older bacterial cultures would not cause disease in chickens **(Fig. 1–1).**[2,3] Subsequent injections of more virulent organisms had no effect on the birds that had been previously exposed to the older cultures. In this manner, the first attenuated vaccine was discovered; this event can be considered the birth of immunology.[3] **Attenuation,** or change, means to make a pathogen less virulent; it takes place through heat, aging, or chemical means. Attenuation remains the basis for many of the immunizations that are used today. Pasteur applied this same principle of attenuation to the prevention of rabies in affected individuals.

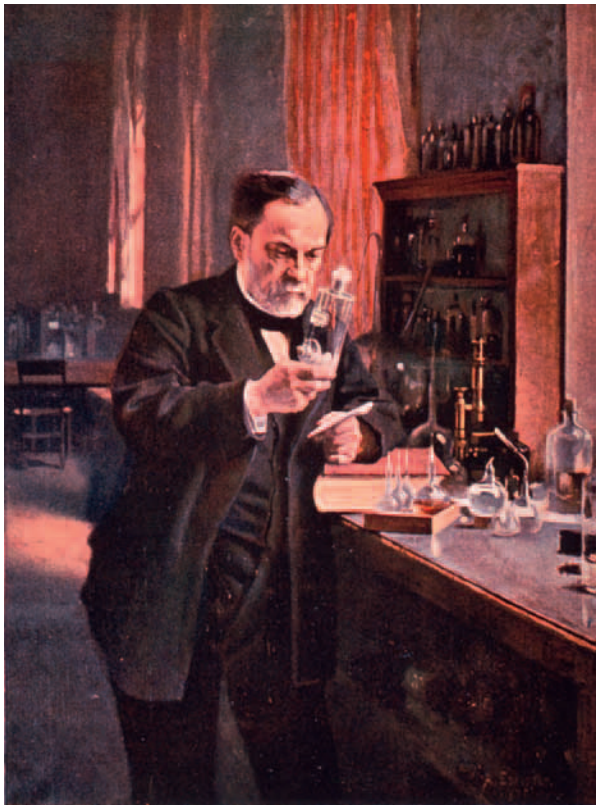

FIGURE 1–1 Louis Pasteur. *(Courtesy of the National Library of Medicine.)*

Innate Versus Adaptive Immunity

In the late 1800s, scientists turned to identifying the actual mechanisms that produce immunity in a host.[2] Elie Metchnikoff, a Russian scientist, observed under a microscope that foreign objects introduced into transparent starfish larvae became surrounded by motile amoeboid-like cells that attempted to destroy the penetrating objects. This process was later termed **phagocytosis,** meaning cells that eat cells.[2,4] He hypothesized that immunity to disease was based on the action of these scavenger cells and was a natural, or innate, host defense.[4]

Other researchers contended that noncellular elements in the blood were responsible for protection from microorganisms. Emil von Behring demonstrated that diphtheria and tetanus toxins, which are produced by specific microorganisms as they grow, could be neutralized by the noncellular portion of the blood of animals previously exposed to the microorganisms. The theory of **humoral immunity** was thus born and sparked a long-lasting dispute over the relative importance of cellular versus humoral immunity.

In 1903, an English physician named Almroth Wright linked the two theories by showing that the immune response involved both cellular and humoral elements. He observed that certain humoral, or circulating, factors called *opsonins* acted to coat bacteria so that they became more susceptible to ingestion by phagocytic cells.[2] These serum factors include specific proteins known as antibodies, as well as other factors called *acute-phase reactants* that increase nonspecifically in any infection. **Antibodies** are serum proteins produced by certain lymphocytes when exposed to a foreign substance and they react specifically with that foreign substance (see Chapter 5).

These discoveries showed that there were two major branches of immunity, currently referred to as innate immunity and adaptive immunity. **Innate,** or **natural immunity,** is the individual's ability to resist infection by means of normally present body functions. These are considered nonadaptive or nonspecific and are the same for all pathogens or foreign substances to which one is exposed. No prior exposure is required and the response lacks memory and specificity. Many of these mechanisms are subject to influence by such factors as nutrition, age, fatigue, stress, and genetic determinants. **Adaptive immunity,** in contrast, is a type of resistance that is characterized by specificity for each individual pathogen, or microbial agent, and the ability to remember a prior exposure. Memory and specificity result in an increased response to that pathogen upon repeated exposure, something that does not occur in innate immunity. Both systems are necessary to maintain good health. In fact, they operate in combination and are dependent upon one another for maximal effectiveness. Certain key cells are considered essential to both systems and they will be discussed next.

Cells of the Innate Immune System

Leukocytes in Peripheral Blood

White blood cells (WBCs), or **leukocytes,** in the peripheral blood play a key role in both innate and adaptive immunity. There are five principal types of leukocytes in peripheral blood: neutrophils, eosinophils, basophils, monocytes, and lymphocytes. The first four types are all part of innate immunity. Because lymphocytes are considered part of adaptive immunity, they will be considered in a separate section. Several cell lines that are found in the tissues, namely mast cells, macrophages, and dendritic cells, will also be discussed in this chapter because they all contribute to the process of immunity.

All blood cells arise from a type of cell called a hematopoietic stem cell (HSC). To form WBCs, the HSC gives rise to two distinct types of precursor cells: common myeloid precursors (CMP) and common lymphoid precursors (CLP). CMPs give rise to the WBCs that participate in phagocytosis, which are known as the myeloid line. Phagocytic cells are key to innate immunity, but they are also important in processing antigens for the adaptive response. Lymphocytes arise from CLPs and form the basis of the adaptive immune response. Mature lymphocytes are found in the tissues as well as in peripheral blood. Refer to **Figure 1–2** for a simplified scheme of blood cell development, known as *hematopoiesis*.

Neutrophils

The **neutrophil,** or polymorphonuclear neutrophilic (PMN) leukocyte, represents approximately 50% to 75% of the total peripheral WBCs in adults.[5] These are around 10 to 15 μm in diameter with a nucleus that has between two and five lobes **(Fig. 1–3).** Hence, they are often called segmented neutrophils, or "segs." They contain a large number of neutral staining granules when stained with Wright stain, two-thirds of which are specific granules; the remaining one-third are called azurophilic granules.[6] Azurophilic or primary granules contain antimicrobial products such as myeloperoxidase, lysozyme, elastase, proteinase-3, cathepsin G, and defensins, which are small proteins that have antibacterial activity.[5] Specific granules, also known as secondary granules, contain lysozyme, lactoferrin, collagenase, gelatinase, and respiratory burst components.[5,7] See Chapter 3 for a discussion of the oxidative burst, which takes place during phagocytosis. The main function of neutrophils is phagocytosis, resulting in the destruction of foreign particles.[6]

Normally, half of the total neutrophil population in peripheral blood is found in a marginating pool adhering to blood vessel walls, whereas the rest circulate freely for approximately 6 to 8 hours.[5] There is a continuous interchange, however, between the marginating and the circulating pools. Margination occurs to allow neutrophils to move from the circulating blood to the tissues through a process known as **diapedesis,** or movement through blood vessel walls. They are attracted to a specific area by chemotactic factors. **Chemotaxins** are chemical messengers that cause cells to migrate in a particular direction. Once in the tissues, neutrophils have a life span of up to several days. Normally, the influx of neutrophils from the bone marrow equals the output from the blood to the tissues to maintain a steady state. However, in the case of acute infection an increase of neutrophils in the circulating blood can occur almost immediately.[8]

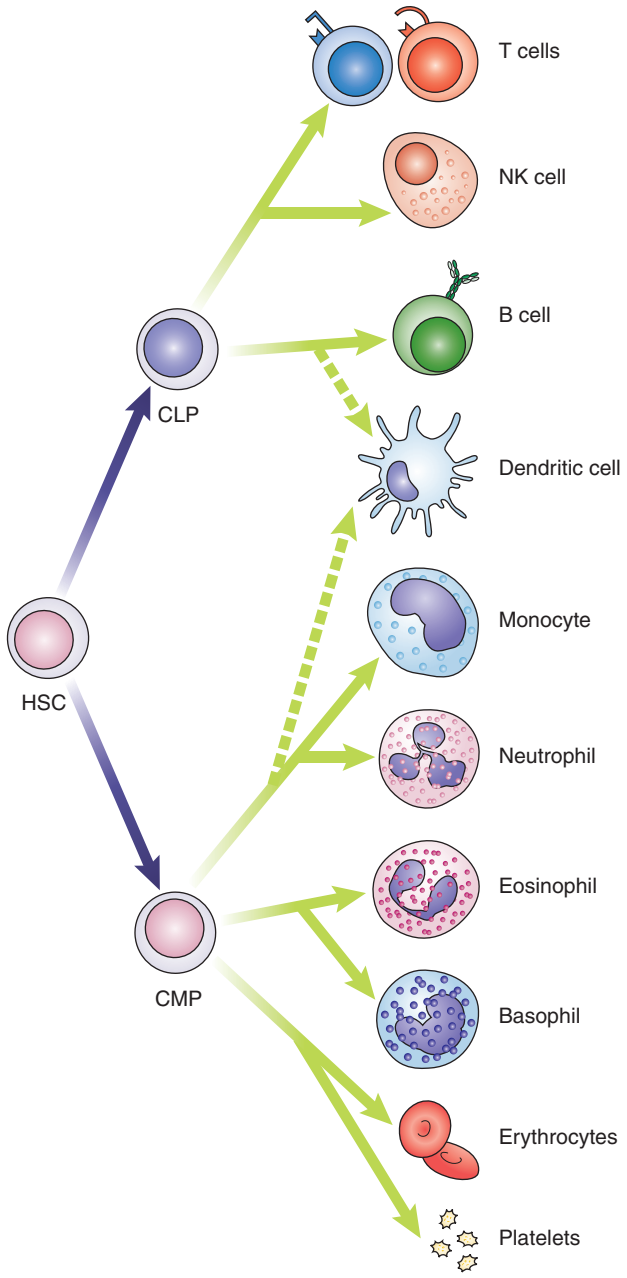

FIGURE 1–2 Simplified scheme of hematopoiesis. In the marrow, hematopoietic stem cells (HSC) give rise to two different lines—a common lymphoid precursor (CLP) and a common myeloid precursor (CMP). CLPs give rise to T/NK progenitors, which differentiate into T and NK cells, and to B-cell progenitors, which become B cells and dendritic cells. The CMP differentiates into neutrophils, monocytes/macrophages, eosinophils, basophils, erythrocytes, and platelets.

Eosinophils

Eosinophils are approximately 12 to 15 μm in diameter and normally make up between 1% and 3% of the circulating WBCs in a nonallergic person. Their number increases in an allergic reaction or in response to certain parasitic infections. The nucleus is usually bilobed or ellipsoidal and is often eccentrically located **(Fig. 1–4).** Eosinophils take up the acid eosin dye and the cytoplasm is filled with large orange to

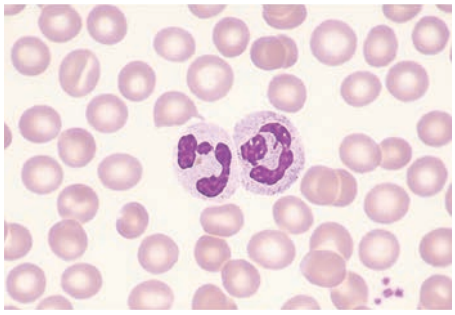

FIGURE 1–3 Neutrophils. *(From Harmening D.* Clinical Hematology and Fundamentals of Hemostasis. *5th ed. Philadelphia, PA: F.A. Davis; 2009. Fig. 1–4.)*

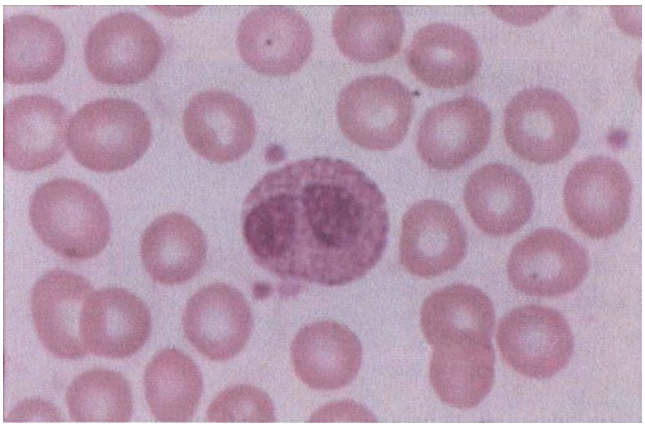

FIGURE 1–4 Eosinophil. *(From Harmening D.* Clinical Hematology and Fundamentals of Hemostasis. *5th ed. Philadelphia, PA: F.A. Davis; 2009. Fig. 1–6.)*

reddish-orange granules. Granules in eosinophils, which are spherical and evenly distributed throughout the cell, contain a large number of previously synthesized proteins including catalase, lysozyme, cytokines (chemical messengers), growth factors, and cationic proteins.[5,9]

Eosinophils are capable of phagocytosis but are much less efficient than neutrophils because they are present in smaller numbers and they lack digestive enzymes. Eosinophils are able to neutralize basophil and mast cell products. In addition, they can use cationic proteins to damage cell membranes and kill larger parasites that cannot be phagocytized. (See Chapter 22 for details.) However, the most important role of eosinophils is regulation of the immune response, including regulation of mast cell function.[5]

Basophils

Basophils are the least numerous of WBCs found in peripheral blood, representing less than 1% of all circulating WBCs. The smallest of the granulocytes, basophils are slightly larger than RBCs (between 10 to 15 μm in diameter) and contain coarse, densely staining deep-bluish-purple granules that often obscure the nucleus[5,9] **(Fig. 1–5).** Constituents of these granules include histamine, cytokines, growth factors, and a small amount of heparin, all of which have an important function in inducing and maintaining allergic reactions.[5,8] Histamine contracts smooth muscle and heparin is

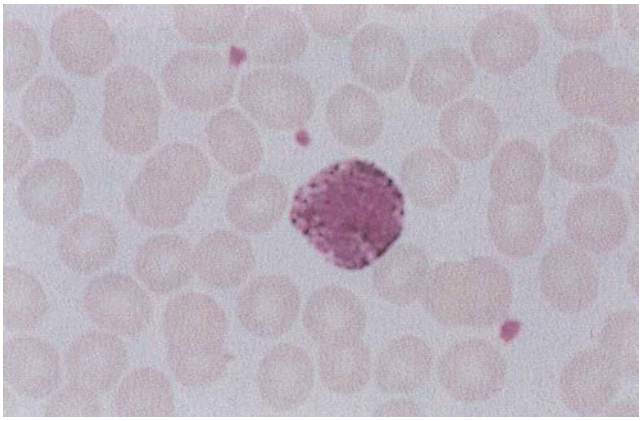

FIGURE 1–5 Basophil. *(From Harmening D.* Clinical Hematology and Fundamentals of Hemostasis. *5th ed. Philadelphia, PA: F.A. Davis; 2009. Fig. 1–7.)*

an anticoagulant. In addition, basophils regulate some T helper (Th) cell responses and stimulate B cells to produce the antibody IgE.[5,10] Basophils have a short life span of only a few hours in the bloodstream; they are then pulled out and destroyed by macrophages in the spleen.

Monocytes

Monocytes are the largest cells in the peripheral blood with a diameter that can vary from 12 to 22 μm (the average is 18 μm).[9] One distinguishing feature is an irregularly folded or horseshoe-shaped nucleus that occupies almost one-half of the entire cell's volume **(Fig. 1–6).** The abundant cytoplasm stains a dull grayish blue and has a ground-glass appearance because of the presence of fine dustlike granules. These granules are actually of two types. The first type contains peroxidase, acid phosphatase, and arylsulfatase, indicating that these granules are similar to the lysosomes of neutrophils.[8] The second type of granule may contain β-glucuronidase, lysozyme, and lipase, but no alkaline phosphatase. Digestive vacuoles may also be observed in the cytoplasm. Monocytes make up between 4% and 10% of total circulating WBCs; however, they do not remain in the circulation for long. They stay in peripheral blood for up to 30 hours; they then migrate to the tissues and become known as macrophages.[5]

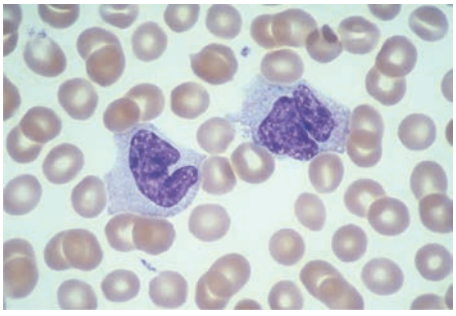

FIGURE 1–6 Two monocytes. *(From Harmening D.* Clinical Hematology and Fundamentals of Hemostasis. *5th ed. Philadelphia, PA: F.A. Davis; 2009. Fig. 1–13.)*

Tissue Cells

Macrophages

All **macrophages** arise from monocytes, which can be thought of as macrophage precursors because additional differentiation and cell division takes place in the tissues. The transition from monocyte to macrophage in the tissues is characterized by progressive cellular enlargement to between 25 and 80 μm.[8] Unlike monocytes, macrophages contain no peroxidase.[8] Tissue distribution appears to be a random phenomenon.

Macrophages have specific names according to their particular tissue location. Macrophages in the lung are alveolar macrophages; in the liver, Kupffer cells; in the brain, microglial cells; in the bone, osteoclasts; and in connective tissue, histiocytes. Macrophages may not be as efficient as neutrophils in phagocytosis because their motility is slow compared with that of the neutrophils. Some macrophages progress through the tissues by means of amoeboid action, whereas others are immobile. However, their life span appears to be in the range of months rather than days.

Macrophages play an important role in initiating and regulating both innate and adaptive immune responses. Their innate immune functions include microbial killing, anti-tumor activity, intracellular parasite eradication, phagocytosis, and secretion of cell mediators. Killing activity is enhanced when macrophages become "activated" by contact with microorganisms or with chemical messengers called **cytokines,** which are released by T lymphocytes during the immune response. (See Chapter 6 for a complete discussion of cytokines.) Macrophages play a major role in the adaptive immune response by presenting antigens to T and B cells.

Mast Cells

Tissue **mast cells** resemble basophils, but they come from a different lineage. Mast cells are distributed throughout the body in a wide variety of tissues such as skin, connective tissue, and the mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts.[5] Mast cells are larger than basophils with a small round nucleus and more granules **(Fig. 1–7).** Unlike basophils, they have a long life span of between 9 and 18 months.[11] The

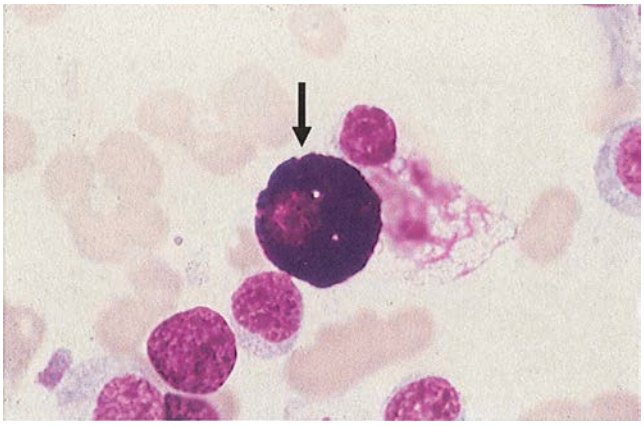

FIGURE 1–7 Mast cell. *(From Harmening D.* Clinical Hematology and Fundamentals of Hemostasis. *5th ed. Philadelphia, PA: F.A. Davis; 2009. Fig. 1–44.)*

enzyme content of the granules in mast cells helps to distinguish them from basophils because they contain acid phosphatase, alkaline phosphatase, and protease, as well as histamine.[5,7,8] Mast cells play a role in allergic reactions, but they can also function as antigen-presenting cells (APCs). They can both enhance and suppress the adaptive immune response.

Dendritic Cells

Dendritic cells are so named because they are covered with long membranous extensions that make them resemble nerve cell dendrites. They were discovered by Steinman and Cohn in 1973.[7] Progenitors in the bone marrow give rise to dendritic cell precursors that travel to lymphoid as well as nonlymphoid tissue.[12] They are classified according to their tissue location in a similar manner to macrophages. After capturing an antigen in the tissue by phagocytosis or endocytosis, dendritic cells present the antigen to T lymphocytes to initiate the adaptive immune response in a similar way as macrophages. Dendritic cells, however, are considered the most effective APC in the body, as well as the most potent phagocytic cell.[13,14]

Cells of the Adaptive Immune System

The key cell involved in the adaptive immune response is the **lymphocyte.** Lymphocytes represent between 20% and 40% of the circulating WBCs. The typical small lymphocyte is similar in size to RBCs (7–10 μm in diameter) and has a large rounded nucleus that may be somewhat indented. The nuclear chromatin is dense and tends to stain a deep blue **(Fig. 1–8).**[9] Cytoplasm is sparse, containing few organelles and no specific granules, and consists of a narrow ring surrounding the nucleus.[6] The cytoplasm stains a lighter blue. These cells are unique because they arise from an HSC and then are further differentiated in the primary lymphoid organs, namely the bone marrow and the thymus. Lymphocytes can be divided into three major populations—T cells, B cells, and natural killer (NK) cells—based on specific functions and the proteins on their cell surfaces. In the peripheral blood of adults, approximately 10% to 20% of lymphocytes are B cells, 61% to 80% are T cells, and 10% to 15% are NK cells.[13]

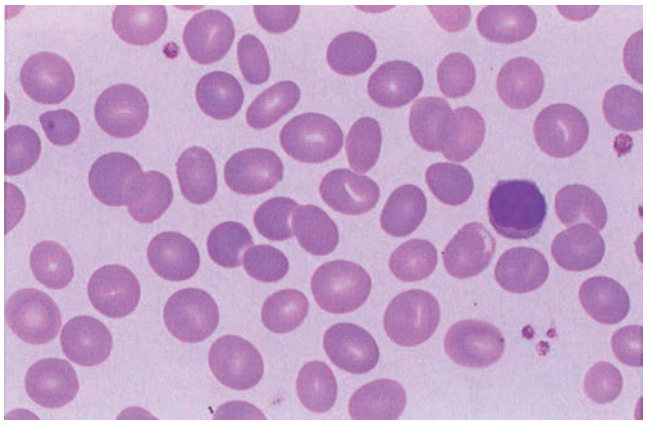

FIGURE 1–8 Typical lymphocyte found in peripheral blood. *(From Harr R.* Clinical Laboratory Science Review. *4th ed. Philadelphia, PA: F.A. Davis; 2013. Color Plate 31.)*

The three types of cells are difficult to distinguish visually. In the laboratory, proteins, or antigens, on cell surfaces can be used to identify each lymphocyte subpopulation. In order to standardize the nomenclature, scientists set up the Human Leukocyte Differentiation Antigens Workshops to relate research findings.[15] Panels of antibodies from different laboratories were used for analysis and antibodies reacting similarly with standard cell lines were said to define **clusters of differentiation (CD).** As each antigen, or CD, was found, it was assigned a number. The list of CD designations currently numbers more than 500.[16] **Table 1–1** lists some of the most important CD numbers used to identify lymphocytes.

B Cells

B cells are derived from a lymphoid precursor that differentiates to become either a T cell, B cell, or NK cell depending on exposure to different cytokines. B cells remain in the environment provided by bone marrow stromal cells. B-cell precursors go through a developmental process that prepares them for their role in antibody production and, at the same time, restricts the types of antigens to which any one cell can respond. The end result is a B lymphocyte programmed to produce a unique antibody molecule. B cells can be recognized by the presence of membrane-bound antibodies of two types, namely immunoglobulin M (IgM) and immunoglobulin (IgD). Other surface proteins that appear on the B cell include CD19, CD21, and class II major histocompatibility complex (MHC) molecules (see Chapter 2).[10]

T Cells

T cells are so named because they differentiate in the thymus. Lymphocyte precursors called **thymocytes** enter the thymus from the bone marrow through the bloodstream. As they mature, the T cells express unique surface markers that allow them to recognize foreign antigens bound to cell membrane proteins called MHC molecules. The role of T cells is to produce cytokines that contribute to immunity by stimulating B cells to produce antibodies, assisting in killing tumor cells or infected target cells, and helping to regulate both the innate and adaptive immune response. The process is known as **cell-mediated immunity.**

Three main subtypes of T cells can be distinguished according to their unique functions: helper, cytolytic, and regulatory T cells. The subtypes can be identified by the presence of the CD3 marker on their cell surface, and either CD4, or CD8. T cells bearing the CD4 receptor are mainly either helper or regulatory cells, whereas the CD8-positive (CD8+) population consists of cytotoxic T cells. The ratio of CD4+ to CD8+ cells is approximately 2:1 in peripheral blood.

Natural Killer (NK) Cells

A small percentage of lymphocytes do not express the markers of either T cells or B cells. They are named **natural killer**

Table 1–1 Surface Markers on T, B, and NK Cells

ANTIGEN	MOL WT (KD)	CELL TYPE	FUNCTION
CD3	20–28	Thymocytes, T cells	Found on all T cells; associated with T-cell antigen receptor
CD4	55	T helper cells, monocytes, macrophages	Identifies T helper cells; also found on most T regulatory cells
CD8	60–76	Thymocyte subsets, cytotoxic T cells	Identifies cytotoxic T cells
CD16	50–80	Macrophages, NK cells, neutrophils	Low affinity Fc receptor for antibody; mediates phagocytosis
CD19	>120	B cells, follicular dendritic cells	Part of B-cell coreceptor; regulates B-cell development and activation
CD21	145	B cells, follicular dendritic cells	Receptor for complement component C3d; part of B-cell coreceptor with CD19
CD 56	175–220	NK cells, subsets of T cells	Not known

(NK) cells because they have the ability to kill target cells without prior exposure to them. NK cells do not require the thymus for development but appear to mature in the bone marrow itself.[17,18] NK cells are generally larger than T cells and B cells at approximately 15 μm in diameter and contain kidney-shaped nuclei with condensed chromatin and prominent nucleoli. Described as large granular lymphocytes, NK cells make up 10% to 15% of the circulating lymphoid pool and are found mainly in the liver, spleen, and peripheral blood.[5,10]

There are no surface markers that are unique to NK cells, but they express a specific combination of antigens that can be used for identification. Two such antigens are CD16 and CD56. CD16 is a receptor for the nonspecific end of antibodies. (See Chapter 5 for more details.) Because of the presence of CD16, NK cells are able to make contact with and then lyse any cell coated with antibodies.[10] NK cells are also capable of recognizing any foreign cell and represent the first line of defense against virally infected cells and tumor cells.[19]

Although NK cells have traditionally been considered part of the innate immune system because they can respond to a variety of antigens, it appears that they also have the capability to develop memory to specific antigens in a similar manner to T cells.[19] Normally, NK cells have a half-life of 7 to 10 days, but new evidence suggests that they are able to survive for a longer time because they can generate highly specific memory cells.[19,20] Thus, they play an important role as a transitional cell bridging the innate and the adaptive immune response against pathogens.[17]

Organs of the Immune System

Just as the cells of the immune system have diverse functions, so, too, do key organs that are involved in the development of the immune response. The bone marrow and thymus are considered the **primary lymphoid organs** where maturation of B lymphocytes and T lymphocytes takes place, respectively. The secondary organs provide a location where contact with foreign antigens can occur **(Fig. 1–9). Secondary lymphoid organs** include the spleen, lymph nodes, and various types of mucosal-associated lymphoid tissues (MALT). The primary and secondary organs are differentiated according to their function in both adaptive and innate immunity.

Primary Lymphoid Organs

Bone Marrow

Bone marrow is considered one of the largest tissues in the body and it fills the core of all long flat bones. It is the main source of hematopoietic stem cells, which develop into erythrocytes, granulocytes, monocytes, platelets, and lymphocytes. Each of these lines has specific precursors that originate from the pleuripotential stem cells.

Some lymphocyte precursors remain in the marrow to mature and become NK and B cells. B cells received their name because they were originally found to mature in birds in an organ called the bursa of Fabricius, which is similar to the appendix in humans. After searching for such an organ in humans, it was discovered that B-cell maturation takes place within the bone marrow itself. Thus, the naming of these cells was appropriate. Other lymphocyte precursors go to the thymus and develop into T cells, so named because of where they mature.[7] Immature T cells appear in the fetus as early as 8 weeks in the gestational period.[21] Thus, differentiation of lymphocytes appears to take place very early in fetal development and is essential to acquisition of immunocompetence by the time the infant is born.

Thymus

T cells develop their identifying characteristics in the **thymus,** which is a small, flat, bilobed organ found in the thorax, or chest cavity, right below the thyroid gland and overlying the heart. In humans, the thymus reaches a weight of 30 to 40 g by puberty and then gradually shrinks in size.[22] It was first thought that the thymus produces enough virgin T lymphocytes early in life to seed the entire immune system, making the organ unnecessary later on. However, it now appears that

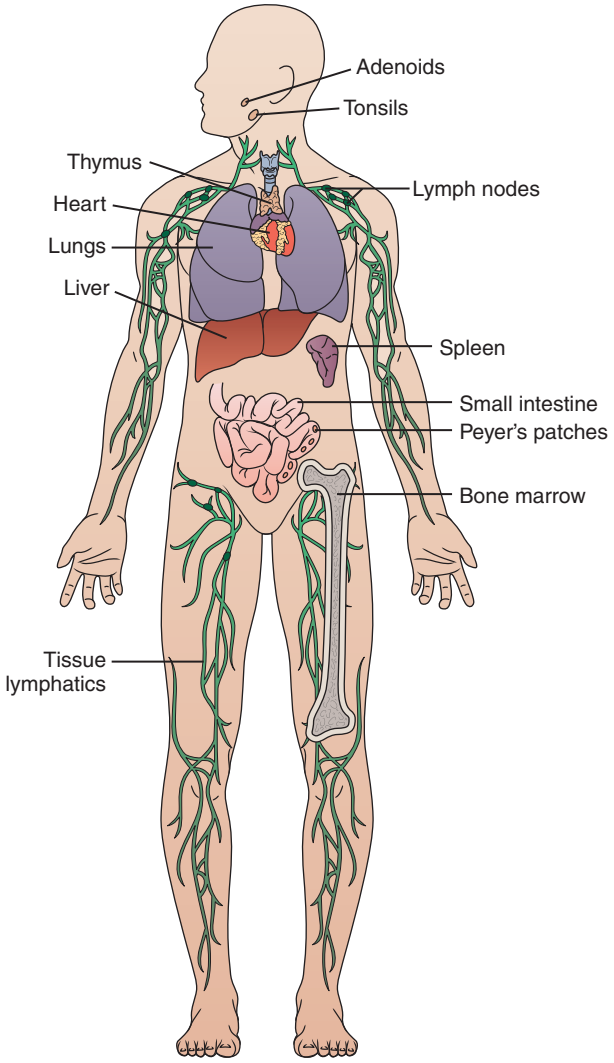

FIGURE 1–9 Sites of lymphoreticular tissue. Primary organs include the bone marrow and the thymus. Secondary organs are distributed throughout the body and include the spleen, lymph nodes, and mucosal-associated lymphoid tissue (MALT). The spleen filters antigens in the blood, whereas the lymphatic system filters fluid from the tissues.

although the thymus diminishes in size as humans age, it is still capable of producing T lymphocytes, although at a diminished rate.[22,23]

Each lobe of the thymus is divided into smaller lobules filled with epithelial cells that play a central role in the differentiation process. Maturation of T cells takes place over a 3-week period as cells filter through the thymic cortex to the medulla. Different surface antigens are expressed as T cells mature. In this manner, a repertoire of T cells is created to protect the body from foreign invaders. Mature T lymphocytes are then released from the medulla.

Secondary Lymphoid Organs

Once lymphocytes mature in the primary organs, they are released and make their way to secondary lymphoid organs, which include the spleen, lymph nodes, cutaneous-associated lymphoid tissue (CALT), and MALT in the respiratory, gastrointestinal, and urogenital tracts. It is within these secondary organs that the main contact with foreign antigens takes place. Lymphocyte circulation between the secondary organs is complex and is regulated by different cell surface adhesion molecules and by cytokines.

Each lymphocyte spends most of its life span in solid tissue, entering the circulation only periodically to go from one secondary organ to another. Lymphocytes in these organs travel through the tissue and return to the bloodstream by way of the thoracic duct. The thoracic duct is the largest lymphatic vessel in the body. It collects most of the body's lymph fluid and empties it into the left subclavian vein. The majority of circulating lymphocytes are T cells.[5] Continuous recirculation increases the likelihood of a T lymphocyte coming into contact with the specific antigen with which it can react.

Lymphocytes are segregated within the secondary organs according to their particular functions. T lymphocytes are effector cells that serve a regulatory role, whereas B lymphocytes produce antibodies. It is in the secondary organs that contact with foreign antigens is most likely to take place.

Lymphopoiesis, or multiplication of lymphocytes, occurs in the secondary lymphoid tissue and is strictly dependent on antigenic stimulation. Formation of lymphocytes in the bone marrow, however, is antigen-independent, meaning that lymphocytes are constantly being produced without the presence of specific antigens. Most naïve or resting lymphocytes die within a few days after leaving the primary lymphoid organs unless activated by the presence of a specific foreign antigen. Antigen activation gives rise to long-lived memory cells and shorter-lived effector cells that are responsible for the generation of the immune response.

Spleen

The **spleen,** the largest secondary lymphoid organ, has a length of approximately 12 cm and weighs 150 g in the adult. It is located in the upper-left quadrant of the abdomen just below the diaphragm and is surrounded by a thin connective tissue capsule. The organ can be characterized as a large discriminating filter as it removes old and damaged cells and foreign antigens from the blood.

Splenic tissue can be divided into two main types: red pulp and white pulp. The red pulp makes up more than one-half of the total volume and its function is to destroy old red blood cells (RBCs). Blood flows from the arterioles into the red pulp and then exits by way of the splenic vein. The white pulp comprises approximately 20% of the total weight of the spleen and contains the lymphoid tissue, which is arranged around arterioles in a **periarteriolar lymphoid sheath (PALS) (Fig. 1–10).** This sheath contains mainly T cells. Attached to the sheath are **primary follicles,** which contain B cells that are not yet stimulated by antigens. Surrounding the PALS is a marginal zone containing dendritic cells that trap antigens. Lymphocytes enter and leave this area by means of the many capillary branches that connect to the arterioles. The spleen receives a blood volume of approximately 350 mL/minute, which allows lymphocytes and macrophages to constantly survey for infectious agents or other foreign matter.[22]