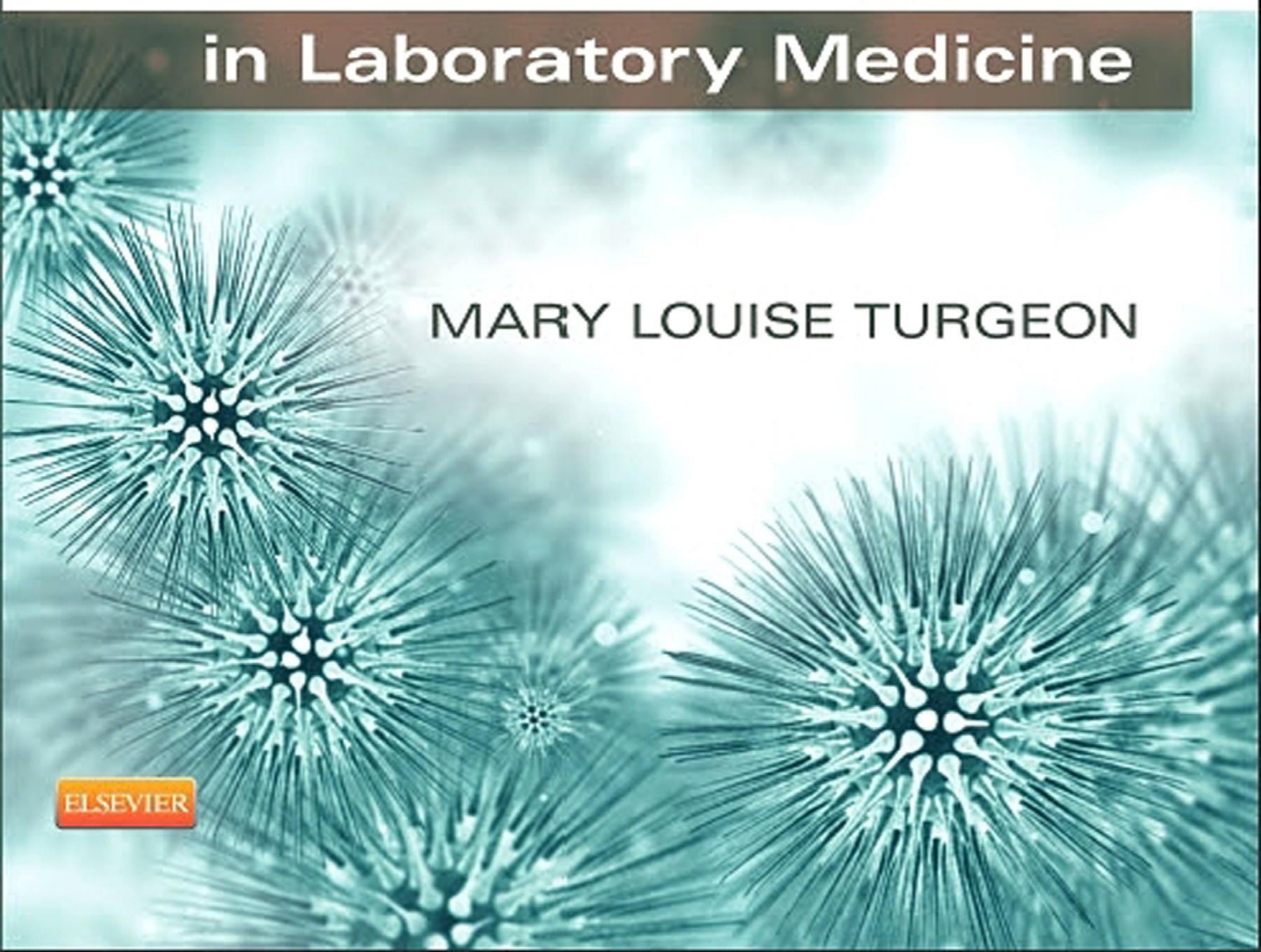
FIFTH EDITION

Immunology & Serology in Laboratory Medicine



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FIFTH EDITION

Immunology & Serology in Laboratory Medicine

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PREFACE

The principles and practice of immunology and serology affect every aspect of the clinical laboratory. Immunology and serology have come to represent the bedrock of laboratory diagnostics by underlying principles or practical applications.

The intention of this fifth edition of *Immunology and Serology in Laboratory Medicine* is to continue to fulfill the needs of medical laboratory technician (MLT) and medical laboratory science (MLS) students and their instructors for an entry-level text that encompasses the most current theory, practice, and clinical applications in the fields of immunology and serology. This textbook is written specifically for students and practitioners in clinical laboratory science.

Content delivery is competency-based to provide the framework for theory and practice, with a strong emphasis on clinical applications. Critical thinking is essential and has a renewed emphasis in this edition, with many more clinical case studies. Every chapter has applicable cases with extensively developed presentations, case-related multiple-choice questions, and critical analysis group discussion questions. These cases not only promote critical thinking and stimulate an overall interest in medicine, but highlight the essential role of the laboratory in patient diagnosis and treatment.

The organization of the book allows for tremendous flexibility in instructional design and delivery. The book is well suited for traditional on-campus instruction, hybrid or blended modes of teaching, and online delivery of courses. A new category of content is the emphasis on Internet-delivered references to sites for virtual laboratories and for the enhancement of learning the content presented in the book.

Students in the digital age are becoming more visual learners. Extensive use is made of new and highly acclaimed illustrations originally published in the *New England Journal of Medicine,* as well as classic presentations from highly regarded immunology reference books. This adds a contemporary and exciting flair to a traditional college textbook. To accommodate student preference for visual presentation of information, the learning experience is enhanced with links to video animations and other digital resources in the textbook, on the Evolve website, and on the author's website. More tables and boxes have been added to chapters.

Each chapter has the principle and clinical application of at least one related procedure. In some cases, this provides the requisite information for a course. The procedural protocol, including specimen collection, the required materials, actual procedure, and expected reference results, are published on the Evolve websites for students and instructors who wish to select that laboratory exercise in their curriculum. Instructors can easily select procedures and create a customized laboratory manual that students can print, as needed. The benefits include reduction in the risk of soiling or contaminating their textbook in a wet laboratory. By reducing the number of pages devoted to laboratory procedures in the text, which may not be desired in a course, the planet gets a little greener with associated savings in the cost of production. Because the diversity in immunology and serology laboratory delivery ranges from a full semester of student laboratories to courses without any on-campus student laboratories, the new edition of this book is linked to a variety of virtual laboratories.

ORGANIZATION

The major topical areas are organized into four primary sections. The entire content of the book has been reviewed and updated with the newest technical and clinical information. Content of the book represents the basic knowledge required for certification examinations for MLT- and MLS-level graduates. Beyond basic knowledge and skills requirements, the text presents interdisciplinary topics and niche topics of transplantation and tumor immunology.

Parts I and II provide foundational knowledge and skills that progress from basic immunologic mechanisms and serologic concepts to the theory of laboratory procedures, including molecular techniques. Parts III and IV emphasize medical applications of importance to clinical laboratory science. In addition, they contain representative disorders of infectious and immunologic origin, as well as topics such as transplantation and tumor immunology. The sequence of the parts has been designed to accommodate the core needs of clinical laboratory students in basic concepts, the underlying theory of procedures, and immunologic manifestations of infectious diseases. Because the needs of some students are more advanced in immunopathology, these topics are presented later in the text to allow students to analyze, evaluate abnormalities, and exercise critical thinking skills based on their knowledge of the preceding parts. Students may study specific components of the text, depending on the level, length, and objectives of the course.

DISTINCTIVE FEATURES AND LEARNING AIDS

As an individually authored textbook, unlike edited books with multiple contributors, students gain the advantage of consistency in writing style and format from chapter to chapter. This fifth edition of *Immunology and Serology in Laboratory Medicine* capitalizes on the strengths of previous editions, beginning with the first edition in 1990.

To address the needs of new learners, key terms and expanded glossary are featured in this edition.

- Key words and a topical outline are presented at the beginning of each chapter. These outlines should be of value to students in the organization of the material and may be of convenience to instructors in preparing lectures.
- The latest illustrations, photographs, and summary tables are used to clarify various conceptual themes and information visually.
- Chapter highlights and review questions are provided at the conclusion of each chapter.
- Additional fully developed clinical case studies, with detailed answers to questions and more review questions, have been added to this edition.

To streamline this text, the principles and clinical applications of representative procedures appear in every chapter of the text. Complete procedural protocols, organized according

to the format suggested by the Clinical Laboratory Standards Institute (CLSI), appear in the online Evolve website.

NEW TO THIS EDITION

What is significant that is new in this fifth edition? The knowledge base in the field of immunology and serology continues to expand logarithmically.

Every chapter has been reviewed and analyzed by clinical laboratory science students and instructors and has been updated, as needed. Each chapter has at least one relevant case study and reference to at least one related procedure. Suggestions for web-based videos and virtual laboratories have been compiled by chapter and presented on the Evolve site.

- In Part I, recent advances in medicine related to inflammation and T lymphocytes have been added, as well as representative procedures.
- Part II, "The Theory of Immunologic and Serologic Procedures," has been enhanced. Representative procedures have been added. The chapter on molecular diagnostics (see Chapter 14) continues to expand because of the increasing emphasis on this method of testing.
- In Part III, a unique chapter, Chapter 16, "A Primer on Vaccines," has been expanded as the importance of vaccines continues to become more evident. This chapter is unique and not available in competing textbooks. Representative case studies have been added to the chapters in this section.
- Part IV, "Immune Disorders," presents the latest information related to transplantation. In addition, information related to tumor immunology has been revised.

Although the content of immunology continues to expand, *Immunology and Serology in Laboratory Medicine* is written for clinical laboratory students in immunology who need an emphasis on the medical aspects of the discipline and the practical aspects of serology. The fifth edition should provide students with a basic foundation in the theory and practice of clinical immunology and practical serology in a one- or two-term course at MLT or MLS levels of instruction.

ANCILLARIES

For the Instructor

Evolve

The companion Evolve website offers several features to aid instructors:

- **Critical Analysis Group Discussion Questions:** Complete explanations are on the instructor's side of Evolve for the open-ended, case-related discussion questions.
- **Test Bank:** This is a test bank of more than 990 multiple choice questions that feature answers, explanations, and cognitive levels. The test bank can be used as review in class or for test development. More than 330 of the questions in the instructor test bank are available for student use.
- **PowerPoint Presentations:** One PowerPoint presentation is given per chapter; this feature can be used as is or as a template to prepare lectures.
- **Image Collection:** All the images from the book are available as *.jpg* files and can be downloaded into PowerPoint presentations. The figures can be used during lectures to illustrate important concepts.
- **Case Studies:** Case studies are provided for additional opportunities for student application of chapter content in real-life scenarios.
- **Procedures:** This feature presents the priniciples and application of procedures in every chapter.
- **Sample syllabi for MLT and MLS Students:** One- and two-semester courses are available.
- **Answers to Additional Review Questions:** Students have access to more than 330 questions that test their knowledge on the concepts presented in the text. The questions and answers are available to instructors.
- **Chapter-linked Digital Enrichment References:** References to videos, animations, and virtual laboratories are available.

For the Student

Evolve

The student resources on Evolve include the following:

- **Additional Review Questions:** A set of more than 330 multiple choice questions provides extra review and practice.

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Dr. Turgeon is the author of medical laboratory science books (sold in more than 45 countries):

- *Immunology and Serology in Laboratory Medicine*, fifth edition (2014)
- *Linné C Ringsrud's Clinical Laboratory Science*, sixth edition (2012)
- *Clinical Hematology*, fifth edition (2012)
- *Fundamentals of Immunohematology*, second edition (1995)

Immunology and Serology in Laboratory Medicine has been translated into Italian and Chinese. *Clinical Hematology* has been translated into Spanish. Dr. Turgeon is the author of numerous professional journal articles.

The presentation of professional workshops and lectures complement the author's teaching and writing activities. Her consulting practice, Mary L. Turgeon and Associates (www.mlturgeon.com), focuses on new program development, curriculum revision, and increasing teaching effectiveness through the use of technology.

Dr. Turgeon's career in medical laboratory science has spanned the globe. Her professional involvement has offered her the opportunity to meet and collaborate with medical laboratory science colleagues in the United States and worldwide, including China, Italy, Japan, Qatar, Saudi Arabia, and the United Arab Emirates. Professional volunteer activities have taken her to Cambodia and Lesotho, Africa.



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PART I

Basic Immunologic Mechanisms

1 An Overview of Immunology
2 Antigens and Antibodies
3 Cells and Cellular Activities of the Immune System: Granulocytes and Mononuclear Cells
4 Cells and Cellular Activities of the Immune System: Lymphocytes and Plasma Cells
5 Soluble Mediators of the Immune System

CHAPTER 1

An Overview of Immunology

History of Immunology
What Is immunology?
Cells of the Immune System
Function of Immunology
Body Defenses: Resistance to Microbial Disease
First Line of Defense
Second Line of Defense: Natural Immunity
Third Line of Defense: Adaptive Immunity
Comparison of Innate and Adaptive Immunity
Pathogen-Associated Molecular Patterns and Pattern Recognition Receptors
Case Study
Questions
Critical Thinking Group Discussion Questions
Procedure: Identification of Leukocytes Related to Immune Function
Chapter Highlights
Review Questions
Bibliography

Learning Objectives

At the conclusion of this chapter, the reader should be able to:

- Compare an immunogen and an antigen
- Define the term *immunology.*
- Explain the functions of the immune system.
- Describe the first, second, and third lines of body defense against microbial diseases.
- Compare innate and adaptive immunity.
- Analyze a case study related to immunity.
- Correctly answer case study related multiple choice questions.
- Be prepared to participate in a discussion of critical thinking questions.
- Describe the characteristics of five mature leukocytes and their immune function.
- Correctly answer end of chapter review questions.

Key Terms

acquired immunity
active immunity
adaptive immune system
allografts
antibodies
antigen
autoimmune disorder
cell-mediated immunity
complement
cytokines
endogenous
exogenous
genome
hematopoietic cells
humoral-mediated immunity
immunocompetent
immunoglobulins
immunology
inflammation
innate immune system
innate resistance
interleukins
major histocompatibility complex (MHC)
mononuclear phagocyte system
nonself
passive immunity
pathogen-associated molecular patterns (PAMPs)
pattern recognition receptors (PRRs)
phagocytosis
vaccination

HISTORY OF IMMUNOLOGY

The science of immunology arose from the knowledge that those who survived one of the common infectious diseases of the past rarely contracted the disease again. As early as 430 BC, during the plague in Athens, Thucydides recorded that individuals who had previously contracted the disease recovered and he recognized their "immune" status.

Beginning about 1000 AD, the Chinese practiced a form of immunization by inhaling dried powders derived from the crusts of smallpox lesions. In the 15th century, powdered smallpox "crusts" were inserted with a pin into the skin. When this practice became popular in England, it was discouraged at first, partly because the practice of inoculation occasionally killed or disfigured a patient.

Louis Pasteur is generally considered to be the Father of Immunology. Table 1-1 lists some historic benchmarks in immunology.

WHAT IS IMMUNOLOGY?

Immunology is defined as resistance to disease, specifically infectious disease. Immunology consists of the following: the study of the molecules, cells, organs, and systems responsible for the recognition and disposal of foreign **(nonself)** material; how body components respond and interact; the desirable and undesirable consequences of immune interactions; and the ways in which the immune system can be advantageously manipulated to protect against or treat disease (Box 1-1). Immunologists in the Western Hemisphere generally exclude from the study of immunology the relationship among cells during embryonic development.

The immune system is composed of a large complex set of widely distributed elements, with distinctive characteristics. Specificity and memory are characteristics of lymphocytes (see Chapter 4). Various specific and nonspecific elements of the immune system demonstrate mobility, including T and B lymphocytes, **immunoglobulins** (antibodies), complement, and **hematopoietic cells.**

CELLS OF THE IMMUNE SYSTEM

Cooperation is required for optimal functioning of the immune system. This cooperative interaction involves specific cellular elements, cell products, and nonlymphoid elements.

Cells of the immune system consist of lymphocytes, specialized cells that capture and display microbial antigen, and effector cells that eliminate microbes (see Color Plate 1). The principal functions of the major cell types involved in the immune response are as follows:

Box 1-1 Role of the Immune System

Defending the body against infections
Recognizing and responding to foreign antigens
Defending the body against the development of tumors

Table 1-1 Significant Milestones in Immunology

Date	Scientist(s)	Discovery
1798	Jenner	Smallpox vaccination
1862	Haeckel	Phagocytosis
1880-1881	Pasteur	Live, attenuated chicken cholera and anthrax vaccines
1883-1905	Metchnikoff	Cellular theory of immunity through phagocytosis
1885	Pasteur	Therapeutic vaccination First report of live "attenuated" vaccine for rabies
1890	Von Behring, Kitasata	Humoral theory of immunity proposed
1891	Koch	Demonstration of cutaneous (delayed-type) hypersensitivity
1900	Ehrlich	Antibody formation theory
1902	Portier, Richet	Immediate-hypersensitivity anaphylaxis
1903	Arthus	Arthus reaction of intermediate hypersensitivity
1938	Marrack	Hypothesis of antigen-antibody binding
1944		Hypothesis of allograft rejection
1949	Salk, Sabin	Development of polio vaccine
1951	Reed	Vaccine against yellow fever
1953		Graft-versus-host reaction
1957	Burnet	Clonal selection theory
1957		Interferon
1958-1962		Human leukocyte antigens (HLAs)
1964-1968		T-cell and B-cell cooperation in immune response
1972		Identification of antibody molecule
1975	Köhler	First monoclonal antibodies
1985-1987		Identification of genes for T cell receptor
1986		Monoclonal hepatitis B vaccine
1986	Mosmann	Th1 versus Th2 model of T helper cell function
1996-1998		Identification of toll-like receptors
2001		FOXP3, the gene directing regulatory T cell development
2005	Frazer	Development of human papillomavirus vaccine

- Specific recognition of antigens
- Capture of antigens for display to lymphocytes
- Elimination of antigens

FUNCTION OF IMMUNOLOGY

The function of the immune system is to recognize self from nonself and to defend the body against nonself. Such a system is necessary for survival. The distinction of self from nonself is made by an elaborate, specific recognition system. Specific cellular elements of the immune system include the lymphocytes. The immune system also has nonspecific effector mechanisms that usually amplify the specific functions. Nonspecific components of the immune system include mononuclear phagocytes, polymorphonuclear leukocytes, and soluble factors (e.g., complement).

Nonself substances range from life-threatening infectious microorganisms to a lifesaving organ transplantation. The desirable consequences of immunity include natural resistance, recovery, and acquired resistance to infectious diseases. A deficiency or dysfunction of the immune system can cause many disorders. Undesirable consequences of immunity include allergy, rejection of a transplanted organ, or an **autoimmune disorder,** in which the body's own tissues are attacked as if they were foreign. Over the last decade, a new concept, the danger theory, has challenged the classic self-nonself viewpoint; although popular, it has not been widely accepted by immunologists (see Chapter 4).

BODY DEFENSES: RESISTANCE TO MICROBIAL DISEASE

First Line of Defense

Before a pathogen can invade the human body, it must overcome the resistance provided by the body's first line of defense (Fig. 1-1). The first barrier to infection is unbroken skin and mucosal membrane surfaces. These surfaces are essential in forming a physical barrier to many microorganisms because this is where foreign materials usually first contact the host. Keratinization of the upper layer of the skin and the constant renewal of the skin's epithelial cells, which repairs breaks in the skin, assist in the protective function of skin and mucosal membranes. In addition, the normal flora (microorganisms

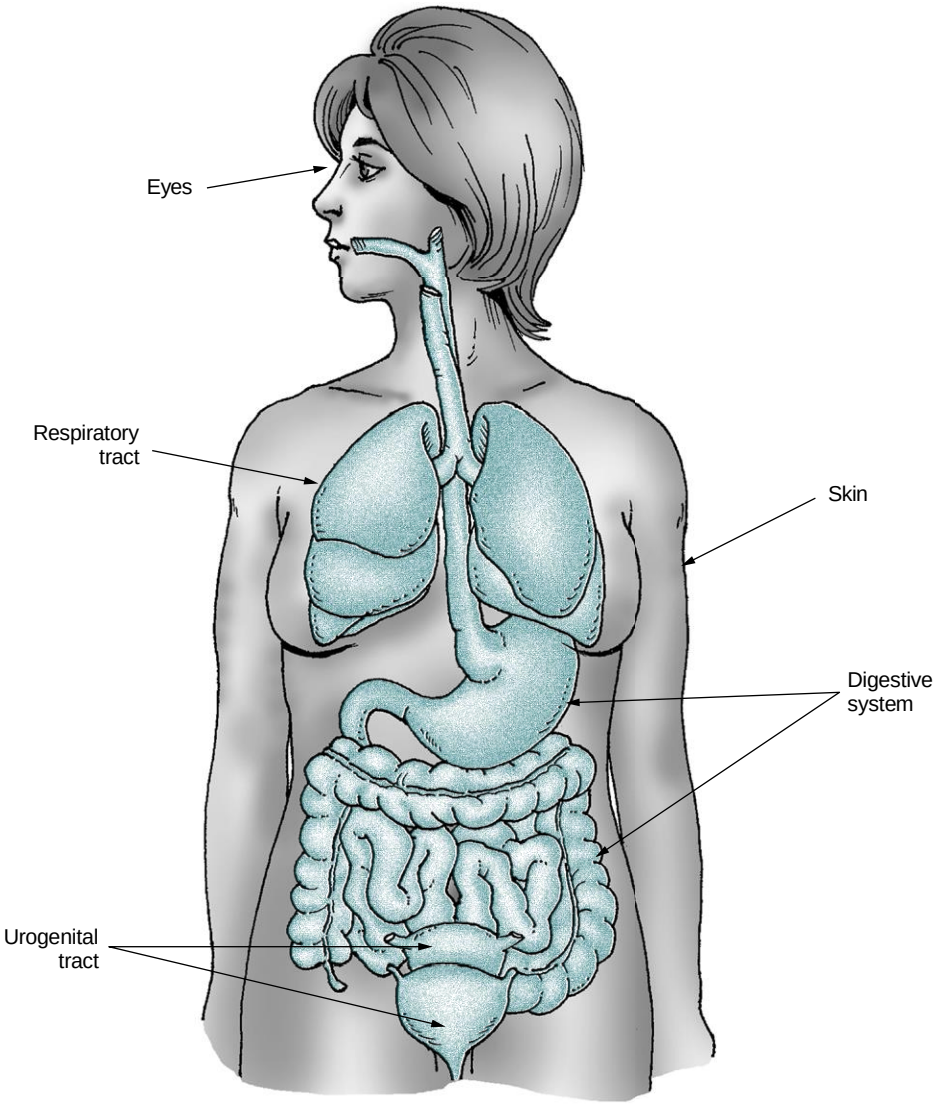

Figure 1-1 First line of defense, nonspecific. Body fluids, specialized cells, fluids, and resident bacteria (normal biota) allow the respiratory, digestive, urogenital, integumentary, and other systems to defend the body against microbial infection.

normally inhabiting the skin and membranes) deter penetration or facilitate elimination of foreign microorganisms from the body.

Secretions are also an important component in the first line of defense against microbial invasion. Mucus adhering to the membranes of the nose and nasopharynx traps microorganisms, which can be expelled by coughing or sneezing. Sebum (oil) produced by the sebaceous glands of the skin and lactic acid in sweat both possess antimicrobial properties. The production of earwax (cerumen) protects the auditory canals from infectious disease. Secretions produced in the elimination of liquid and solid wastes (e.g., urinary and gastrointestinal processes) are important in physically removing potential pathogens from the body. The acidity and alkalinity of the fluids of the stomach and intestinal tract, as well as the acidity of the vagina, can destroy many potentially infectious microorganisms. Additional protection is provided to the respiratory tract by the constant motion of the cilia of the tubules.

In addition to the physical ability to wash away potential pathogens, tears and saliva also have chemical properties that defend the body. The enzyme lysozyme, which is found in tears and saliva, attacks and destroys the cell wall of susceptible bacteria, particularly certain gram-positive bacteria. Immunoglobulin A (IgA) antibody is another important protective substance in tears and saliva.

Second Line of Defense: Natural Immunity

Natural immunity (inborn or **innate resistance**) is one of the ways that the body resists infection after microorganisms have penetrated the first line of defense. Acquired resistance, which specifically recognizes and selectively eliminates exogenous or endogenous agents, is discussed later.

Natural immunity is characterized as a nonspecific mechanism. If a microorganism penetrates the skin or mucosal membranes, a second line of cellular and humoral defense mechanisms becomes operational (Box 1-2). The elements of natural resistance include phagocytic cells, complement, and the acute inflammatory reaction (see Chapter 3).

Detection of microbial pathogens is carried out by sentinel cells of the innate immune system located in tissues (macrophages and dendritic cells [DCs]) in close contact with the host's natural environment or that are rapidly reunited to the site of infection (neutrophils). Despite their relative lack of specificity, these cellular components are essential because they are largely responsible for natural immunity to many environmental microorganisms. These phagocytic cells, which engulf invading foreign material, constitute major cellular components. Tissue damage produced by infectious or other agents results in **inflammation,** a series of biochemical and cellular changes that facilitate **phagocytosis** (engulfment and destruction) of microorganisms or damaged cells (see Chapter 3). If the degree of inflammation is sufficiently extensive, it is accompanied by an increase in the plasma concentration of acute-phase proteins or reactants, a group of glycoproteins. Acute-phase proteins are sensitive indicators of the presence of inflammatory disease and are especially useful in monitoring such conditions (see Chapter 5).

Box 1-2 Components of the Natural Immune System: The Second Line of Defense

Cellular
Mast cells
Neutrophils
Macrophages

Humoral
Complement
Lysozyme
Interferon

Box 1-3 Components of the Adaptive Immune System

Cellular
T lymphocytes
B lymphocytes
Plasma cells

Humoral
Antibodies
Cytokines

Complement proteins are the major humoral (fluid) component of natural immunity (see Chapter 5). Other substances of the humoral component include lysozymes and interferon, sometimes described as natural antibiotics. Interferon is a family of proteins produced rapidly by many cells in response to viral infection; it blocks the replication of virus in other cells.

Third Line of Defense: Adaptive Immunity

If a microorganism overwhelms the body's natural resistance, a third line of defensive resistance exists. Acquired, or adaptive, immunity is a more recently evolved mechanism that allows the body to recognize, remember, and respond to a specific stimulus, an **antigen.** Adaptive immunity can result in the elimination of microorganisms and recovery from disease and the host often acquires a specific immunologic memory.This condition of memory or recall (acquired resistance) allows the host to respond more effectively if reinfection with the same microorganism occurs.

Adaptive immunity, as with natural immunity, is composed of cellular and humoral components (Box 1-3). The major cellular component of **acquired immunity** is the lymphocyte (see Chapter 4); the major humoral component is the antibody (see Chapter 2). Lymphocytes selectively respond to nonself materials (antigens), which leads to immune memory and a permanently altered pattern of response or adaptation to the environment. Most actions in the two categories of the adaptive response, **humoral-mediated immunity** and cell-mediated immunity, are exerted by the interaction of antibody with complement and the phagocytic cells (natural immunity) and of T cells with macrophages (Table 1-2).

Humoral-Mediated Immunity

If specific **antibodies** have been formed to antigenic stimulation, they are available to protect the body against foreign substances. The recognition of foreign substances and subsequent production

Table 1-2	Characteristics of Two Types of Adaptive Immunity	
	Humoral-Mediated Immunity	Cell-Mediated Immunity
Mechanism	Antibody mediated	Cell mediated
Cell type	B lymphocytes	T lymphocytes
Mode of action	Antibodies in serum	Direct cell-to-cell contact or soluble products secreted by cells
Purpose	Primary defense against bacterial infection	Defense against viral and fungal infections, intracellular organisms, tumor antigens, and graft rejection

of antibodies to these substances define immunity. Antibody-mediated immunity to infection can be acquired if the antibodies are formed by the host or if they are received from another source; these two types of acquired immunity are called active immunity and passive immunity, respectively (Table 1-3).

Active immunity can be acquired by natural exposure in response to an infection or natural series of infections, or through intentional injection of an antigen. The latter, **vaccination** (see Chapter 16), is an effective method of stimulating antibody production and memory (acquired resistance) without contracting the disease. Suspensions of antigenic materials used for immunization may be of animal or plant origin. These products may consist of living suspensions of weak or attenuated cells or viruses, killed cells or viruses, or extracted bacterial products (e.g., altered and no longer poisonous toxoids used to immunize against diphtheria and tetanus). The selected agents should stimulate the production of antibodies without clinical signs and symptoms of disease in an **immunocompetent** host (host is able to recognize a foreign antigen and build specific antigen-directed antibodies) and result in permanent antigenic memory. Booster vaccinations may be needed in some cases to expand the pool of memory cells. The mechanisms of antigen recognition and antibody production are discussed in Chapter 2.

Artificial **passive immunity** is achieved by the infusion of serum or plasma containing high concentrations of antibody or lymphocytes from an actively immunized individual. Passive immunity via pre-formed antibodies in serum provides immediate, temporary antibody protection against microorganisms (e.g., hepatitis A) by administering preformed antibodies. The recipient will benefit only temporarily from passive immunity for as long as the antibodies persist in the circulation. Immune antibodies are usually of the IgG type (see Chapter 2, Antigens and Antibodies) with a half-life of 23 days.*

The main strategies for cancer immunotherapy aim to provide antitumor effectors (T lymphocytes and antibodies) to patients. The purpose is to immunize patients actively against their own tumors and to stimulate the patient's own antitumor immune responses.

In addition, passive immunity can be acquired naturally by the fetus through the transfer of antibodies by the maternal placental circulation in utero during the last 3 months of pregnancy (Fig. 1-2). Maternal antibodies are also transferred to the newborn after birth. The amount and specificity of maternal antibodies depend on the mother's immune status to infectious diseases that she has experienced.

Passively acquired immunity in newborns is only temporary because it starts to decrease after the first several weeks or months after birth. Breast milk, especially the thick yellowish milk (colostrum), produced for a few days after the birth of a baby is very rich in antibodies. However, for a newborn to have lasting protection, active immunity must occur.

Cell-Mediated Immunity

Cell-mediated immunity consists of immune activities that differ from antibody-mediated immunity. Lymphocytes are the unique bearers of immunologic specificity, which depends on their antigen receptors. The full development and expression of immune responses, however, require that nonlymphoid cells and molecules primarily act as amplifiers and modifiers.

Cell-mediated immunity is moderated by the link between T lymphocytes and phagocytic cells (i.e., monocytes-macrophages). A B lymphocyte can probably respond to a native antigenic determinant of the appropriate fit. A T lymphocyte responds to antigens presented by other cells in the context of **major histocompatibility complex (MHC)** proteins (see Chapter 31). The T lymphocyte does not directly recognize the antigens of microorganisms or other living cells, such as **allografts** (tissue from a genetically different member of the same species, such as a human kidney), but recognizes when the antigen is present on the surface of an antigen-presenting cell (APC), the macrophage. APCs were at first thought to be limited to cells of the **mononuclear phagocyte system.** Recently, other types of cells (e.g., endothelial, glial) have been shown to possess the ability to present antigens.

Lymphocytes are immunologically active through various types of direct cell-to-cell contact and by the production of soluble factors (see Chapter 5). Nonspecific soluble factors are made by or act on various elements of the immune system. These molecules are collectively called **cytokines.** Some mediators that act between leukocytes are called **interleukins.**

Under some conditions, the activities of cell-mediated immunity may not be beneficial. Suppression of the normal adaptive immune response by drugs or other means is necessary in conditions or procedures such as organ transplantation, hypersensitivity, and autoimmune disorders.

COMPARISON OF INNATE AND ADAPTIVE IMMUNITY

Traditionally, the immune system has been divided into innate and adaptive components, each with a different function and role. The **innate immune system,** an ancient form of host

*Antibody half-life is a measure of the mean survival time of antibody molecules following their formation. It is usually expressed as the time required to eliminate 50% of a known quantity of immunoglobulin from the body. Half-life varies from one immunoglobulin class to another.

Table 1-3 Comparison of Types of Acquired Immunity

	Type	Mode of Acquisition	Antibody Produced by Host	Duration of Immune Response
Active	Natural	Infection	Yes	Long*, †
	Artificial	Vaccination	Yes	Long*, †
Passive	Natural	Transfer in vivo or colostrum	No	Short
	Artificial	Infusion of serum/plasma	No	Short

*Immunocompetent host.
†IgG immune antibody half-life is 23 days. Memory cells (memory lymphocytes) lifespan is years.

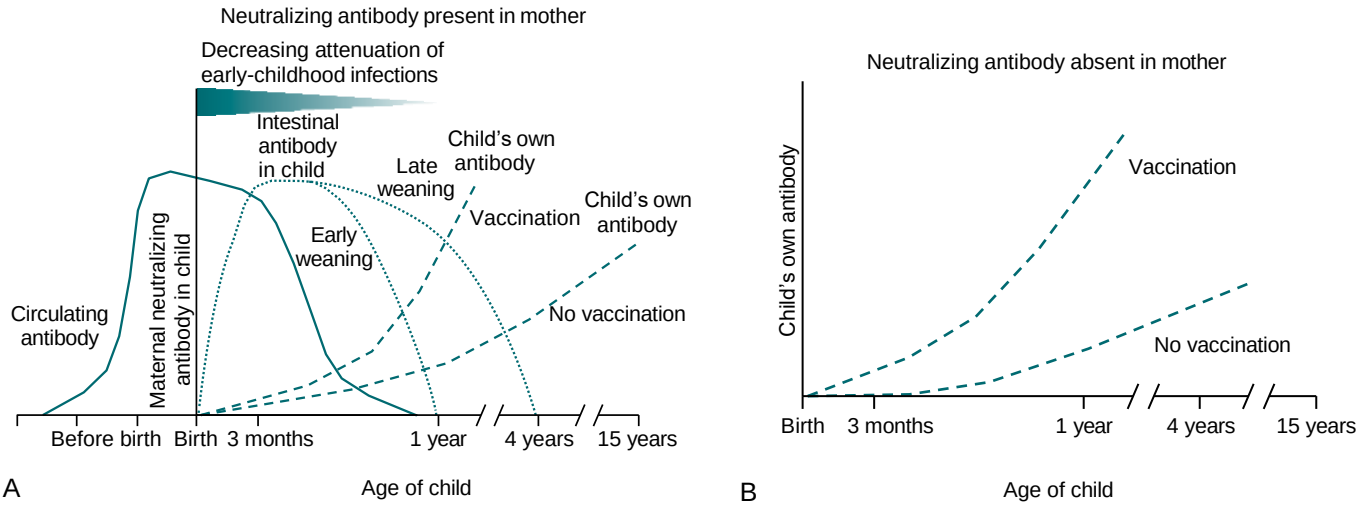

Figure 1-2 Protective effect of maternal antibodies in serum and milk. A, Maternal neutralizing antibodies cross the placenta to protect the offspring and attenuate systemic infections for 6 to 12 months after birth. The timing of weaning, early or late, influences the levels of intestinal antibodies derived from breast milk and the rate of attenuation of gastrointestinal infection. **B,** The absence of specific neutralizing antibodies in maternal serum leads to the absence of a protective effect. *(From Zinkernagel RM: Maternal antibodies, childhood infections, and autoimmune diseases, N Engl J Med 345:1331–1335, 2001.)*

defense, appeared before the adaptive immune system. Mechanisms of innate immunity (e.g., phagocytes) and the alternative complement pathways are activated immediately after infection and quickly begin to control multiplication of infecting microorganisms. By comparison, the **adaptive immune system** (Table 1-4) is organized around two classes of cells, T and B lymphocytes. When an individual lymphocyte encounters an antigen that binds to its unique antigen receptor site, activation and proliferation of that lymphocyte occur. This is called clonal selection and is responsible for the basic properties of the adaptive immune system.

Random generation of a highly diverse database of antigen receptors allows the adaptive immune system to recognize virtually any antigen. The downside to this recognition is the inability to distinguish foreign antigens from self antigens. Activation of the adaptive immune response can be harmful to the host when the antigens are self or environmental antigens. Environmental antigens are epitopes that can be found in infectious microorganisms or dietary sources. They can mimic other antigens and trigger an autoimmune condition.

Some form of innate immunity probably exists in all multicellular organisms. Innate immune recognition is mediated by germline-encoded receptors, which means that the specificity of each receptor is genetically predetermined. Germline-encoded receptors evolved by natural selection to have defined specificities for infectious microorganisms. The problem is that every organism has a limit as to the number of genes it can encode in its **genome.**

Consequently, the innate immune response may not be able to recognize every possible antigen, but may focus on a few large groups of microorganisms, called **pathogen-associated molecular patterns (PAMPs).** The receptors of the innate immune system that recognize these PAMPs are called **pattern recognition receptors** (**PRRs;** e.g., Toll-like receptors).

Pathogen-Associated Molecular Patterns and Pattern Recognition Receptors

PAMPs are molecules associated with groups of pathogens that are recognized by cells of the innate immune system. PRRs are found in plants and animals.

Pattern Recognition Receptors

Three groups of PRRs exist:

1. Secreted PRRs are molecules that circulate in blood and lymph; circulating proteins bind to PAMPs on the surface of many pathogens. This interaction triggers the

Table 1-4 Comparison of Innate and Adaptive Immunity

Innate Immunity	Adaptive Immunity
Pathogen recognized by receptors encoded in the germline	Pathogen recognized by receptors generated randomly
Receptors have broad specificity, i.e., recognize many related molecular structures (PAMPs)	Receptors have very narrow specificity; i.e., recognize a specific epitope
Immediate response	Slow (3-5 days) response
Little or no memory of prior antigenic exposure	Memory of prior antigenic exposure

complement cascade, leading to the opsonization of the pathogen and its speedy phagocytosis (discussed in Chapter 3).

2. Phagocytosis receptors are cell surface receptors that bind the pathogen, initiating a signal leading to the release of effector molecules (e.g., cytokines). Macrophages have cell surface receptors that recognize PAMPs containing mannose.
3. Toll-like receptors (TLRs) are a set of transmembrane receptors that recognize different types of PAMPs. TLRs are found on macrophages, dendritic cells, and epithelial cells.
   - Mammals have multiple TLRs, with each exhibiting a specialized function, frequently with the aid of accessory molecules, in a subset of PAMPs. In this way, TLRs identify the nature of the pathogen and turn on an effector response appropriate for counteracting with it. These signaling cascades lead to the expression of various cytokine genes. Examples include TLR-1, which binds to the peptidoglycan of gram-positive bacteria and TLR-2, which binds lipoproteins of gram-negative bacteria.

In all these cases, binding of the pathogen to the TLR initiates a signaling pathway, leading to the activation of nuclear factor κB (NF-κB, light-chain enhancer of activated B cells). This transcription factor turns on many cytokine genes, such as tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), and chemokines. All these effector molecules lead to the inflammation site (see Chapter 5).

CASE STUDY

A 1-month-old infant female neonate born 6 weeks premature was admitted for surgery to her foot. Several days after hospital discharge, her parents brought her back to the emergency department because she had a high fever and was crying all of the time. Physical examination revealed increased body temperature, increased respiration rate, and increased heart rate. She also had redness around the site of an inserted percutaneous central line related to her surgery.

Her blood count was normal except for a decreased concentration of blood platelets. A smear and a culture were taken from the inflamed area. The direct smears revealed the presence of yeast. Pending results of the culture, the patient was started on antifungal therapeutics. She was admitted to the hospital, where her condition improved within the first 24 hours.

Subsequently, the culture demonstrated *Candida albicans*.

Questions

1. A risk factor for the development of a fungal infection in this child is:
   a. Gender
   b. Body weight
   c. Premature birth
   d. Decreased blood platelet count
2. The child's immune problem is related to:
   a. A lack of immune antibodies to yeast
   b. Defect in her cellular immune response
   c. Lack of sunshine and vitamins
   d. Acquiring the infection from her mother

See Appendix A for the answers to these questions.

Critical Thinking Group Discussion Questions

1. Why is the child at risk for developing an infection of this type?
2. Why did this child acquire an infection?

See Instructor site Evolve for a discussion of the answers to these questions.

Identification of Leukocytes Related to Immune Function

Principle

A whole blood smear is prepared and stained for microscopic examination. Five mature leukocytes with various immune functions can be identified.

See Evolve for a complete discussion of the method.

Results

The specific leukocytes and their related immune functions are as follows:

Band and segmented neutrophils = phagocytosis
Lymphocytes = recognition of foreign antigens and transformation to antibody producing cells
Monocytes = phagocytosis
Eosinophils = allergic reactions
Basophils = anaphylactic reactions

CHAPTER HIGHLIGHTS

- Immunology is defined as the study of the molecules, cells, organs, and systems responsible for the recognition and disposal of nonself material; how body components

respond and interact; desirable or undesirable consequences of immune interactions; and how the immune system can be manipulated to protect against or treat disease.

- The function of the immune system is to recognize self from nonself and to defend the body against nonself.
- The first line of defense against infection is unbroken skin, mucosal membrane surfaces, and secretions.
- Natural immunity consisting of cellular and humoral defense mechanisms forms the second line of body defenses.
- If a microorganism overwhelms the body's natural resistance, a third line of defensive resistance, acquired (or adaptive) immunity, allows the body to recognize, remember, and respond to a specific stimulus, an antigen. Antibody-mediated immunity to infection can be acquired if the antibodies are formed by the host (active immunity) or received from another source (passive immunity).
- Cell-mediated immunity differs from antibody-mediated immunity.
- Lymphocytes are immunologically active through direct cell to cell contact and production of cytokines for specific immunologic functions, such as recruitment of phagocytic cells to the site of inflammation.
- The main difference between the innate and adaptive immune systems is the mechanisms and receptors used for immune recognition.

REVIEW QUESTIONS

1-5. Match the following terms to their appropriate definitions or descriptions. (Use each answer only once.)

1. _____ Immune system
2. _____ Lymphocytes
3. _____ Cooperative interaction
4. _____ Nonspecific immune elements
5. _____ Autoimmune disorder
   a. T and B types
   b. Specific cellular elements, cell products, and nonlymphoid elements
   c. Mononuclear phagocytes
   d. Condition in which the body's own tissues are attacked as if they were foreign
   e. Can protect against or be manipulated to treat disease

6. The first line of defense in protecting the body from infection includes all the following components except:
   a. Unbroken skin
   b. Normal microbial flora
   c. Phagocytic leukocytes
   d. Secretions such as mucus

7. Natural immunity is characterized as being:
   a. Innate or inborn
   b. Able to recognize exogenous or endogenous agents specifically
   c. Able to eliminate exogenous or endogenous agents selectively
   d. Part of the first line of body defenses against microbial organisms

8 and 9. Complete the chart below from the following list of choices:
   a. Lymphocytes
   b. Macrophages
   c. Mucus
   d. Interferons

Components of the Natural Immune System

Cellular	Mast cells Neutrophils 8. _____
Humoral	Complement Lysozyme 9. _____

10. Another term for adaptive immunity is:
    a. Antigenic immunity
    b. Acquired immunity
    c. Lymphocyte reactive immunity
    d. Phagocytosis

11. Humoral components of the adaptive immune system include:
    a. T lymphocytes
    b. B lymphocytes
    c. Antibodies
    d. Saliva

12-23. Complete the table below, choosing from the following answers:

Possible answers for questions 12-15:
   a. Infusion of serum of plasma
   b. Transfer in vivo or by colostrum
   c. Vaccination
   d. Infection

Comparison of the Types of Adaptive Immunity

Type	Mode of Acquisition
Active natural	12. _____
Artificial active	13. _____
Passive natural	14. _____
Artificial passive	15. _____

Comparison of the Types of Adaptive Immunity

Possible answers for questions 16-19:
   a. Yes
   b. No

Type	Antibody Produced by Host
Active natural	16. _____
Artificial active	17. _____
Passive natural	18. _____
Artificial passive	19. _____

Comparison of the Types of Adaptive Immunity

Possible answers for questions 20-23:

a. Short
b. Long

Type	Duration of Response
Active natural	20. _____
Artificial active	21. _____
Passive natural	22. _____
Artificial passive	23. _____

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CHAPTER 2

Antigens and Antibodies

Antigen Characteristics
- General Characteristics of Immunogens and Antigens
- Histocompatibility Antigens
- Autoantigens
- Blood Group Antigens

Chemical Nature of Antigens
- Adjuvant

Physical Nature of Antigens
- Foreignness
- Degradability
- Molecular Weight
- Structural Stability
- Complexity

General Characteristics of Antibodies

Immunoglobulin (Ig) Classes
- Immunoglobulin M
- Immunoglobulin G
- Immunoglobulin A
- Immunoglobulin D
- Immunoglobulin E

Antibody Structure
- Typical Immunoglobulin Molecule
- Fab, Fc, and Hinge Molecular Components
- Structures of Other Immunoglobulins

Immunoglobulin Variants
- Isotype Determinants
- Allotype Determinants
- Idiotype Determinants

Antibody Synthesis
- Primary Antibody Response
- Secondary (Anamnestic) Response

Functions of Antibodies

Antigen-Antibody Interaction: Specificity and Cross-Reactivity
- Antibody Affinity
- Antibody Avidity
- Immune Complexes

Molecular Basis of Antigen-Antibody Reactions
- Types of Bonding
- Goodness of Fit
- Detection of Antigen-Antibody Reactions
- Influence of Antibody Types on Agglutination

Monoclonal Antibodies
- Discovery of the Technique
- Monoclonal Antibody Production
- Uses of Monoclonal Antibodies

Case Study
- Questions
- Critical Thinking Group Discussion Questions

Procedure: ABO Blood Grouping (Forward Antigen Typing)

Procedure: Serum Protein Electrophoresis

Chapter Highlights

Review Questions

Bibliography

Learning Objectives

At the conclusion of this chapter, the reader should be able to:

- Define the terms *antigen* and *antibody.*
- Compare the characteristics of major histocompatibility complex (MHC) classes I and II.
- Name and compare the characteristics of each of the five immunoglobulin classes.
- Draw and describe a typical immunoglobulin G (IgG) molecular structure.
- Name the four phases of an antibody response.
- Describe the characteristics of a primary and secondary (anamnestic) response.
- Compare the terms antibody *avidity* and antibody *affinity.*
- Describe the method of production of a monoclonal antibody.
- Analyze a case study related to antigens or antibodies.
- Correctly answer case study related multiple choice questions.
- Be prepared to participate in a discussion of critical thinking questions.
- Describe the principle and agglutination reactions in ABO blood grouping.
- Describe the principle, expected results, reference values, and clinical interpretation of the serum protein electrophoresis procedure.
- Correctly answer end of chapter review questions.

Key Terms

adjuvant
affinity
alloantibodies
anamnestic response
antibodies
antigens
autoantigen
avidity
clonal selection
epitope
haptens
human leukocyte antigen (HLA)
hybridoma
idiotypes
immune complex
immunogens
major histocompatibility complex (MHC)
monoclonal antibody (MAb)
precipitating
soluble
zeta potential

ANTIGEN CHARACTERISTICS

General Characteristics of Immunogens and Antigens

An immune response is triggered by **immunogens,** macromolecules capable of triggering an adaptive immune response by inducing the formation of **antibodies** or sensitized T cells in an immunocompetent host (a host capable of recognizing and responding to a foreign antigen). Immunogens can specifically react with corresponding antibodies or sensitized T lymphocytes. In contrast, an **antigen** is a substance that stimulates antibody formation and has the ability to bind to an antibody or a T lymphocyte antigen receptor but may not be able to evoke an immune response initially. For example, lower molecular weight particles, haptens, can bind to an antibody but must be attached to a macromolecule as a carrier to stimulate a specific immune response. In reality, all immunogens are antigens but not all antigens are immunogens. The two terms, *immunogens* and *antigens,* are frequently used interchangeably without making a distinction between the two terms.

Foreign substances can be immunogenic or antigenic (capable of provoking a humoral and/or cell-mediated immune response) if their membrane or molecular components contain(s) structures recognized as foreign by the immune system. These structures are called antigenic determinants, or epitopes. An **epitope,** as part of an antigen, reacts specifically with an antibody or T lymphocyte receptor.

Not all surfaces act as antigenic determinants. Only prominent determinants on the surface of a protein are normally recognized by the immune system and some of these are much more immunogenic than others. An immune response is directed against specific determinants and resultant antibodies will bind to them, with much of the remaining molecule being immunogenic.

The cellular membrane of mammalian cells consists chemically of proteins, phospholipids, cholesterol, and traces of polysaccharide. Polysaccharides (carbohydrates) in the form of glycoproteins or glycolipids can be found attached to the lipid and protein molecules of the membrane. When antigen-bearing cells, such as red blood cells (RBCs), from one person, a donor, are transfused into another person, a recipient, they can be immunogenic. Outer surfaces of bacteria, such as the capsule or the cell wall, as well as the surface structures of other microorganisms, can also be immunogenic.

Cellular antigens of importance to immunologists include histocompatibility antigens, autoantigens, and blood group antigens (see later, "ABO Blood Grouping Procedure"). The normal immune system responds to foreignness by producing antibodies. For this reason, microbial antigens are also important to immunologists in the study of the immunologic manifestations of infectious disease.

Histocompatibility Antigens

Nucleated cells such as leukocytes and tissues possess many cell surface–protein antigens that readily provoke an immune response if transferred into a genetically different (allogenic) individual of the same species. Some of these antigens, which constitute the **major histocompatibility complex (MHC)** (see Color Plate 2),

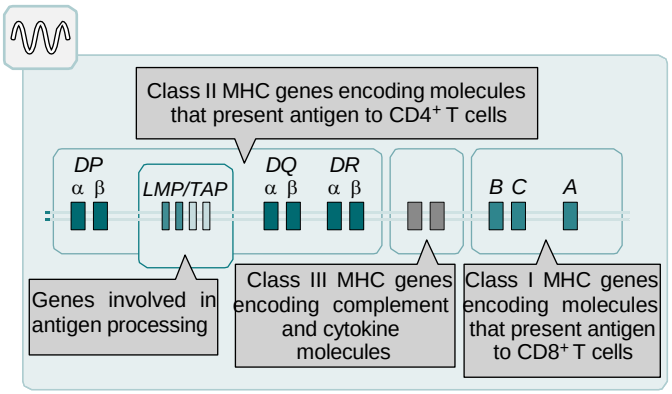


Figure 2-1 Genetic organization of MHC (HLA) antigen. *LMP,* Large multifunctional protease; *TAP,* transporter associated with antigen presentation. *(From Nairn R, Helbert M: Immunology for medical students, ed 2, St Louis, 2007, Mosby.)*

Table 2-1 Comparison of MHC Class I and Class II

	Class I	Class II
Loci	HLA-A, -B, and -C	HLA-DN, -DO, -DP, -DQ, and -DR
Distribution	Most nucleated cells	B lymphocytes, macrophages, other antigen-presenting cells, activated T lymphocytes
Function	To present endogenous antigen to cytotoxic T lymphocytes	To present endogenous antigen to helper T lymphocytes

are more potent than others in provoking an immune response. The MHC is referred to as the **human leukocyte antigen (HLA)** system in humans because its gene products were originally identified on white blood cells (WBCs, leukocytes). These antigens are second only to the ABO antigens in influencing the survival or graft rejection of transplanted organs. HLAs are the subject of numerous scientific investigations because of the strong association between individual HLAs and immunologic disorders (see Chapter 31 for more discussion of the MHC).

Major Histocompatibility Complex Regions

The MHC is divided into four major regions (Fig. 2-1)—D, B, C, and A. The A, B, and C regions are the classic or class Ia genes that code for class I molecules. The D region codes for class II molecules. Class I includes HLA-A, HLA-B, and HLA-C. The three principal loci (A, B, and C) and their respective antigens are numbered, for example, as 1, 2, 3. The class II gene region antigens are encoded in the HLA-D region and can be subdivided into three families, HLA-DR, HLA-DC (DQ), and HLA-SB (DP).

Classes of HLA Molecules

Structurally, there are two classes of HLA molecules, class I and class II (Table 2-1). Both class I and class II antigens function as

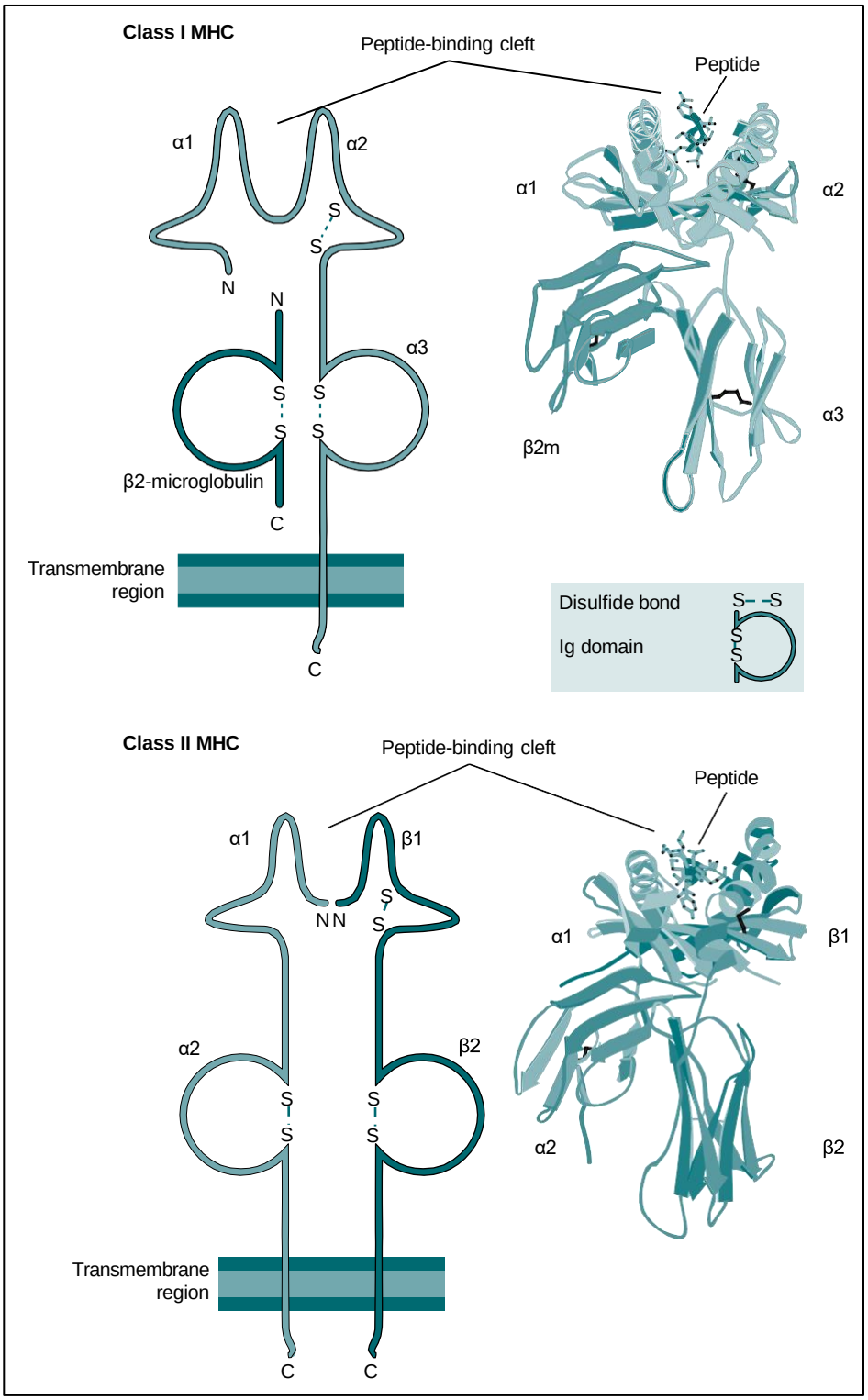


Figure 2-2 Structure of class I and class II MHC molecules. The schematic diagrams *(left)* and models *(right)* of the crystal structures of class I and class II MHC molecules illustrate the domains of the molecules and the fundamental similarities between them. Both types of MHC molecules contain peptide-binding clefts and invariant portions that bind CD8 (the α_3 domain of class I) or CD4 (the β_2 domain of class II). fi_2m, β_2-Microglobulin. *(From Abbas AK, Lichtman AH: Basic immunology: functions and disorders of the immune system, updated edition, ed 3, Philadelphia, 2011, Saunders; crystal structures courtesy Dr. P. Bjorkman, California Institute of Technology, Pasadena, Calif.)*

targets of T lymphocytes (see Chapter 4 for a further discussion of lymphocytes) that regulate the immune response (Fig. 2-2). Class I molecules regulate interaction between cytolytic T cells and target cells and class II molecules restrict the activity of regulatory T cells. Thus, class II molecules regulate the interaction between helper T cells and antigen-presenting cells (APCs). Cytotoxic T cells directed against class I antigens are inhibited by CD8 cells; cytotoxic T cells directed against class II antigens are inhibited by CD4 cells. Many genes in the class I and class II gene families have no known function.

Autoantigens

The evolution of a recognition system that can recognize and destroy nonself material must also have safeguards to prevent damage to self antigens.The body's immune system usually exercises tolerance to self antigens but, in some situations, antibodies

may be produced in response to normal self antigens.This failure to recognize self antigens can result in autoantibodies directed at hormones, such as thyroglobulin (see Chapter 28).

Blood Group Antigens

Blood group substances are widely distributed throughout the tissues, blood cells, and body fluids. When foreign RBC antigens are introduced to a host, a transfusion reaction or hemolytic disease of the fetus and newborn can result (see Chapter 26). In addition, certain antigens, especially those of the Rh system, are integral structural components of the erythrocyte (RBC) membrane. If these antigens are missing, the erythrocyte membrane is defective and results in hemolytic anemia. When antigens do not form part of the essential membrane structure (e.g., A, B, and H antigens), the absence of antigen has no effect on membrane integrity.

CHEMICAL NATURE OF ANTIGENS

Antigens, or immunogens, are usually large organic molecules that are proteins or large polysaccharides and, rarely, if ever, lipids. Antigens, especially cell surface or membrane-bound antigens, can be composed of combinations of biochemical classes (e.g., glycoproteins, glycolipids). For example, histocompatibility HLAs are glycoprotein in nature and are found on the surface membranes of nucleated body cells composed of solid tissue and most circulating blood cells (e.g., granulocytes, monocytes, lymphocytes, thrombocytes).

Proteins are excellent antigens because of their high molecular weight and structural complexity. Lipids are considered inferior antigens because of their relative simplicity and lack of structural stability. However, when lipids are linked to proteins or polysaccharides, they may function as antigens. Nucleic acids are poor antigens because of relative simplicity, molecular flexibility, and rapid degradation. Anti–nucleic acid antibodies can be produced by artificially stabilizing them and linking them to an immunogenic carrier. Carbohydrates (polysaccharides) by themselves are considered too small to function as antigens. In the case of erythrocyte blood group antigens, protein or lipid carriers may contribute to the necessary size and the polysaccharides present in the form of side chains confer immunologic specificity.

Adjuvant

The response to immunization can be enhanced by a number of agents, collectively called adjuvants. One of the best-known emulsifying agents in vaccine studies is Freund's complete adjuvant. An **adjuvant** is a substance, distinct from antigen, that enhances T cell activation by promoting the accumulation of APCs at a site of antigen exposure and by enhancing the expression of costimulators and cytokines by the APCs.

PHYSICAL NATURE OF ANTIGENS

Important factors in the effective functioning of antigens include foreignness, degradability, molecular weight (MW), structural stability, and complexity.

Foreignness

Foreignness is the degree to which antigenic determinants are recognized as nonself by an individual's immune system. The immunogenicity of a molecule depends to a great extent on its degree of foreignness. For example, if a transplant recipient receives a donor organ with several major HLA differences, the organ is perceived as foreign and is subsequently rejected by the recipient. Normally, an individual's immune system does not respond to self antigens.

Degradability

For an antigen to be recognized as foreign by an individual's immune system, sufficient antigens to stimulate an immune response must be present. Foreign molecules are rapidly destroyed and thus cannot provide adequate antigenic exposure. In the case of vaccination, an adequate dose of vaccine at appropriate intervals must be administered for an immune response to be stimulated.

Molecular Weight

The higher the MW, the better the molecule will function as an antigen. The number of antigenic determinants on a molecule is directly related to its size. For example, proteins are effective antigens because of a large MW.

Although large foreign molecules (MW 10,000 daltons [Da]) are better antigens, **haptens,** which are tiny molecules, can bind to a larger carrier molecule and behave as antigens. If a hapten is chemically linked to a large molecule, a new surface structure is formed on the large molecule, which may function as an antigenic determinant.

Structural Stability

If a molecule is an effective antigen, structural stability is mandatory. If a structure is unstable (e.g., gelatin), the molecule will be a poor antigen. Similarly, totally inert molecules are poor antigens. Ther structural stability of an antigen is important in cases where the goal is to elicit a patient antibody response when adminstering a vaccine.

Complexity

The more complex an antigen, the greater is its effectiveness. Complex proteins are better antigens than large repeating polymers such as lipids, carbohydrates, and nucleic acids, which are relatively poor antigens.

GENERAL CHARACTERISTICS OF ANTIBODIES

Antibodies are specific proteins referred to as immunoglobulins. Many antibodies can be isolated in the gamma globulin fraction of protein by electrophoresis separation (Fig. 2-3). The term *immunoglobulin* (Ig) has replaced gamma globulin because not all antibodies have gamma electrophoretic mobility. Antibodies can be found in blood plasma and in many body fluids (e.g., tears, saliva, colostrum).

The primary function of an antibody in body defenses is to combine with antigen, which may be enough to neutralize bacterial toxins or some viruses. A secondary interaction of an

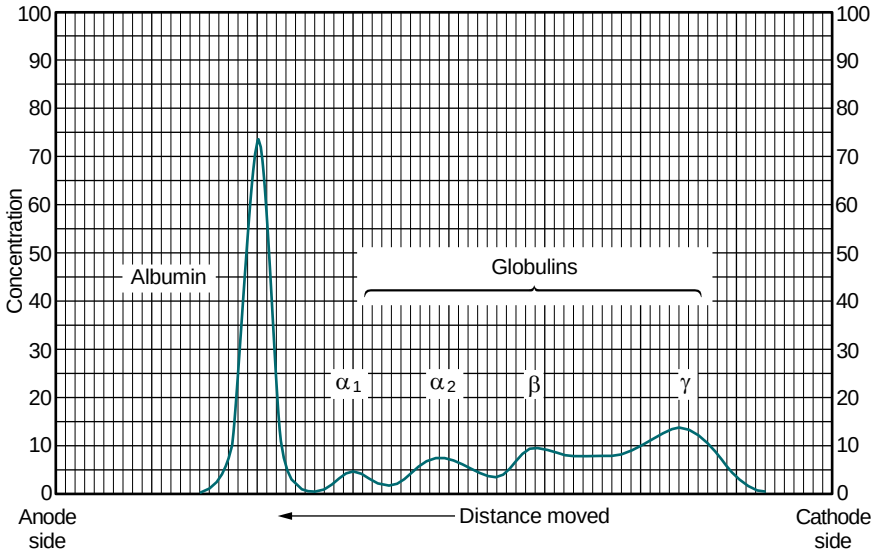


Figure 2-3 Tracing of the electrophoretic pattern of normal serum. *(Adapted from Kaplan LA, Pesce AJ, Kazmierczak SC, editors: Clinical chemistry: theory, analysis, correlation, ed 4, St Louis, 2003, Mosby.)*

Table 2-2 Characteristics of Immunoglobulin Classes

	IgM	IgG	IgA	IgE	IgD
Molecular weight (daltons, Da)	900,000	160,000	360,000	200,000	160,000
Sedimentation coefficient (Σ)	19	7	11	8	7
Carbohydrate (%)	12	8	7	12	12
Subclasses	—	IgG1-4	α1, α2	—	—
Serum concentration, adults (mg/mL)	1.5	13.5	3.5	0.05	Trace
Serum half-life (days)*	5	23	6	2.5	3

*Half life (days) = the amount of time to reach ½ activity concentration. Serum values are average concentrations in normal, healthy individuals. Adapted from Peakman M, Vergani D: Basic and clinical immunology, St Louis, 2009, Elsevier, p 41.

antibody molecule with another effector agent (e.g., complement) is usually required to dispose of larger antigens (e.g., bacteria).

Determining Ig concentration can be of diagnostic significance in infectious and autoimmune diseases. Test methods to detect the presence and concentration of immunoglobulins are discussed in Part II and in chapters relating to specific diseases.

IMMUNOGLOBULIN (IG) CLASSES

Five distinct classes of immunoglobulin molecules are recognized in most higher mammals—IgM, IgG, IgA, IgD, and IgE. These Ig classes differ from each other in characteristics such as MW and sedimentation coefficients (Table 2-2).

Immunoglobulin M

Immunoglobulin M accounts for about 10% of the Ig pool and is largely confined to the intravascular pool because of its large size. This antibody is produced early in an immune response and is largely confined to the blood. IgM is effective in agglutination and cytolytic reactions. In humans, IgM is found in smaller concentrations than IgG or IgA. The molecule has five individual heavy chains, with an MW of 65,000 Da; the whole molecule has an MW of 900,000 Da and sedimentation coefficient, Σ, of 19.

Normal values of IgM are 60 to 250 mg/dL (70 to 290 IU/mL) for males and 70 to 280 mg/dL (80 to 320 IU/mL) for females. At 4 months of age, 50% of the adult level is present; adult levels are reached by 8 to 15 years. Cord blood contains greater than 20 mg/dL. IgM is usually undetectable in cerebrospinal fluid (CSF).

IgM is decreased in primary (genetically determined) Ig disorders as well as secondary Ig deficiencies (acquired disorders associated with certain diseases). IgM can be increased in the following conditions:

- Infectious diseases, such as subacute bacterial endocarditis, infectious mononucleosis, leprosy, trypanosomiasis, malaria, and actinomycosis
- Collagen disorders, such as scleroderma
- Hematologic disorders, such as polyclonal gammopathies, monocytic leukemia, and monoclonal gammopathies (e.g., Waldenström's macroglobulinemia)

Immunoglobulin G

The major immunoglobulin in normal serum is IgG. It diffuses more readily than other immunoglobulins into the extravascular spaces and neutralizes toxins or binds to microorganisms in extravascular spaces. IgG can cross the placenta. In addition, when IgG complexes are formed, complement can be activated. IgG accounts for 70% to 75% of the total Ig pool. It is a 7S molecule, with an MW of approximately 150,000 Da. One of the subclasses, IgG3, is slightly larger (170,000 Da) than the other subclasses.

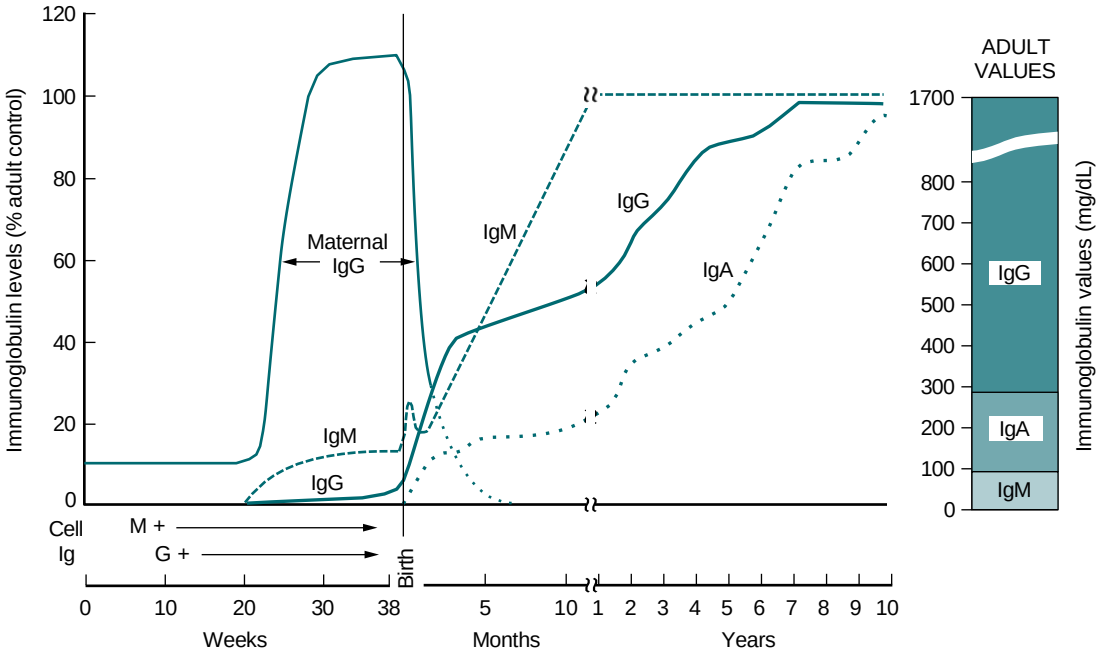


Figure 2-4 Immunoglobulin concentration in newborns, infants, and children. *(Adapted from Bauer JD: Clinical laboratory methods, ed 9, St Louis, 1982, Mosby.)*

Normal human adult serum values of IgG are 800 to 1800 mg/dL (90 to 210 IU/mL). In infants 3 to 4 months old, the IgG level is approximately 350 to 400 mg/dL (40 to 45 IU/mL), gradually increasing to 700 to 800 mg/dL (80 to 90 IU/mL) by the end of the first year of life (Fig. 2-4). The average adult level is achieved before age 16 years. Other body fluids containing IgG include cord blood (800 to 1800 mg/dL) and CSF (2 to 4 mg/dL).

Decreased levels of IgG can be manifested in primary (genetic) or secondary (acquired) Ig deficiencies. Significant increases of IgG are seen in the following conditions:

- Infectious diseases, such as hepatitis, rubella, and infectious mononucleosis
- Collagen disorders, such as rheumatoid arthritis and systemic lupus erythematosus
- Hematologic disorders, such as polyclonal gammopathies, monoclonal gammopathies, monocytic leukemia, and Hodgkin's disease

Immunoglobulin A

Immunoglobulin A represents 15% to 20% of the total circulatory Ig pool. It is the predominant immunoglobulin in secretions such as tears, saliva, colostrum, milk, and intestinal fluids. IgA is synthesized largely by plasma cells located on body surfaces. If produced by cells in the intestinal wall, IgA may pass directly into the intestinal lumen or diffuse into the blood circulation. As IgA is transported through intestinal epithelial cells or hepatocytes, it binds to a glycoprotein called the secretory component. The secretory piece protects IgA from digestion by gastrointestinal proteolytic enzymes. It forms a complex molecule termed *secretory IgA*, which is critical in protecting body surfaces against invading microorganisms because of its presence in seromucous secretions (e.g., tears, saliva, nasal fluids, colostrum).

IgA monomer is present in relatively high concentrations in human serum; it has a concentration of 90 to 450 mg/dL (55 to 270 IU/mL) in normal adult humans. At the end of the first year of life, 25% of the adult IgA level is reached, and 50% at 3.5 years of age. The average adult level is attained by age 16 years. IgA concentration in cord blood is greater than 1 mg/dL; CSF contains 0.1 to 0.6 mg/dL of IgA.

IgA is decreased in primary or secondary Ig deficiencies. Significant increases in serum IgA concentration are associated with the following:

- Infectious diseases, such as tuberculosis and actinomycosis
- Collagen disorders, such as rheumatoid arthritis
- Hematologic disorders, such as polyclonal gammopathies, monocytic leukemia, and monoclonal gammopathy (e.g., IgA myeloma)
- Liver disease, such as Laennec's cirrhosis and chronic active hepatitis

Immunoglobulin D

Immunoglobulin D is found in very low concentrations in plasma, accounting for less than 1% of the total Ig pool. IgD is extremely susceptible to proteolysis and is primarily a cell membrane Ig found on the surface of B lymphocytes in association with IgM.

Immunoglobulin E

Immunoglobulin E is a trace plasma protein found in the blood plasma of unparasitized individuals (MW, 188,000 Da). IgE is crucial because it mediates some types of hypersensitivity

(allergic) reactions, allergies, and anaphylaxis and is generally responsible for an individual's immunity to invading parasites. The IgE molecule is unique in that it binds strongly to a receptor on mast cells and basophils and, together with antigen, mediates the release of histamines and heparin from these cells.

ANTIBODY STRUCTURE

Antibodies exhibit diversity among the different classes, which suggests that they perform different functions in addition to their primary function of antigen binding. Essentially, each Ig molecule is bifunctional; one region of the molecule involves binding to antigen, and a different region mediates binding of the immunoglobulin to host tissues, including cells of the immune system and the first component (C1q) of the classic complement system.

The primary core of an antibody consists of the sequence of amino acid residues linked by the peptide bond. All antibodies have a common, basic polypeptide structure, with a three-dimensional configuration. The polypeptide chains are linked by covalent and noncovalent bonds, which produce a unit composed of a four-chain structure based on pairs of identical heavy and light chains. IgG, IgD, and IgE occur only as monomers of the four-chain unit, IgA occurs in both monomeric and polymeric forms, and IgM occurs as a pentamer with five four-chain subunits linked together.

Typical Immunoglobulin Molecule

The basic unit of an antibody structure is the homology unit, or domain. A typical molecule has 12 domains, arranged in two heavy (H) and two light (L) chains, linked through cysteine residues by disulfide bonds so that the domains lie in pairs (Fig. 2-5). The antigen-binding portion of the molecule (N-terminal end) shows such heterogeneity that it is known as the variable (V) region; the remainder is composed of relatively constant amino acid sequences, the constant (C) region. Short segments of about 10 amino acid residues within the variable regions of antibodies (or T cell receptor [TCR] proteins) form loop structures called complementary-determining regions (CDRs). Three hypervariable loops, also called CDRs, are present in each antibody H chain and L chain. Most of the variability among different antibodies or TCRs is located within these loops.

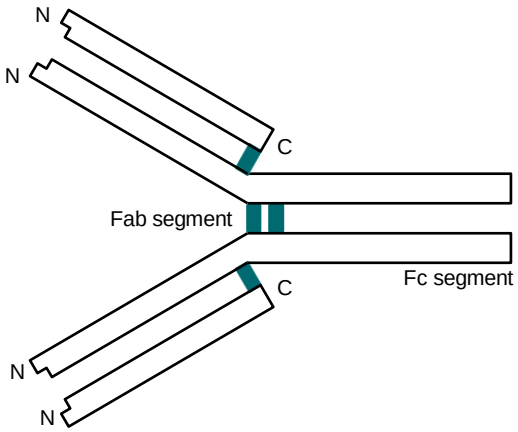


Figure 2-5 Basic immunoglobulin configuration. *(Adapted from Turgeon ML: Fundamentals of immunohematology, ed 2, Baltimore, 1995, Williams & Wilkins.)*

The IgG molecule provides a classic model of antibody structure, appearing Y-shaped under electron microscopy (Fig. 2-6). If the molecule is studies by chemical treatment and the interchain disulfide bonds are broken, the molecule separates into four polypeptide chains. Light chains are small chains (25,000 Da) common to all Ig classes. The L chains are of two subtypes, kappa (κ) and lambda (λ), which have different amino acid sequences and are antigenically different. In humans, about 65% of Ig molecules have κ chains, whereas 35% have λ chains. The larger H chains (50,000 to 77,000 Da) extend the full length of the molecule.

A general feature of the Ig chains is their amino acid sequence. The first 110 to 120 amino acids of both L and H chains have a variable sequence and form the V region; the remainder of the L chains represents the C region, with a similar amino acid sequence for each type and subtype. The remaining portion of the H chain is also constant for each type and has a hinge region. The class and subclass of an Ig molecule are determined by its H-chain type.

Fab, Fc, and Hinge Molecular Components

A typical monomeric IgG molecule consists of three globular regions (two Fab regions and an Fc portion) linked by a flexible hinge region. If the molecule is digested with a proteolytic enzyme such as papain, it splits into three approximately equal-sized fragments (Fig. 2-7). Two of these fragments retain the ability to bind antigen and are called the antigen-binding fragments (Fab fragments). The third fragment, which is relatively homogeneous and is sometimes crystallizable, is called the Fc portion. If IgG is treated with another proteolytic enzyme,

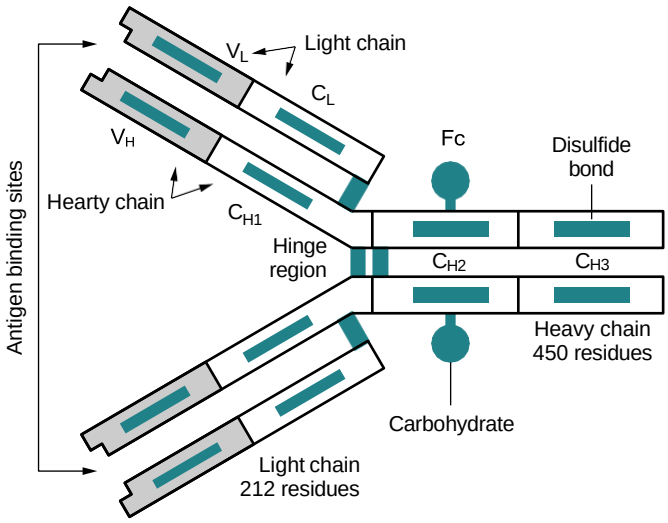


Figure 2-6 Basic structure of IgG. *(Adapted from Turgeon ML: Fundamentals of immunohematology, ed 2, Baltimore, 1995, Williams & Wilkins.)*

pepsin, the molecule separates somewhat differently. The Fc fragment is split into tiny peptides and thus is completely destroyed. The two Fab fragments remain joined to produce a fragment called F(ab)′2. This fragment possesses two antigen-binding sites. If F(ab)′2 is treated to reduce its disulfide bonds, it breaks into two Fab fragments, each of which has only one antigen-binding site. Further disruption of the interchain disulfide bonds in the Fab fragments shows that each contains a light chain and half of a heavy chain, which is called the Fd fragment.

Electron microscopy studies of IgG have revealed that the Fab regions of the molecule are mobile and can swing freely around the center of the molecule as if it were hinged. This hinge consists of a group of about 15 amino acids located between the C_{H1} and C_{H2} regions. The exact sequence of amino acids in the hinge is variable and unique for each Ig class and subclass. Because amino acids can rotate freely around peptide bonds, the effect of closely spaced proline amino acid residues is production of a so-called universal joint, around which the Ig chains can swing freely. A remarkable feature of the hinge region is the presence of a large number of hydrophilic and proline residues. The hydrophilic residues tend to open up this region and thus make it accessible to proteolytic cleavage with enzymes such as pepsin and papain. This region also contains all the interchain disulfide bonds except for IgD, which has no interchain links.

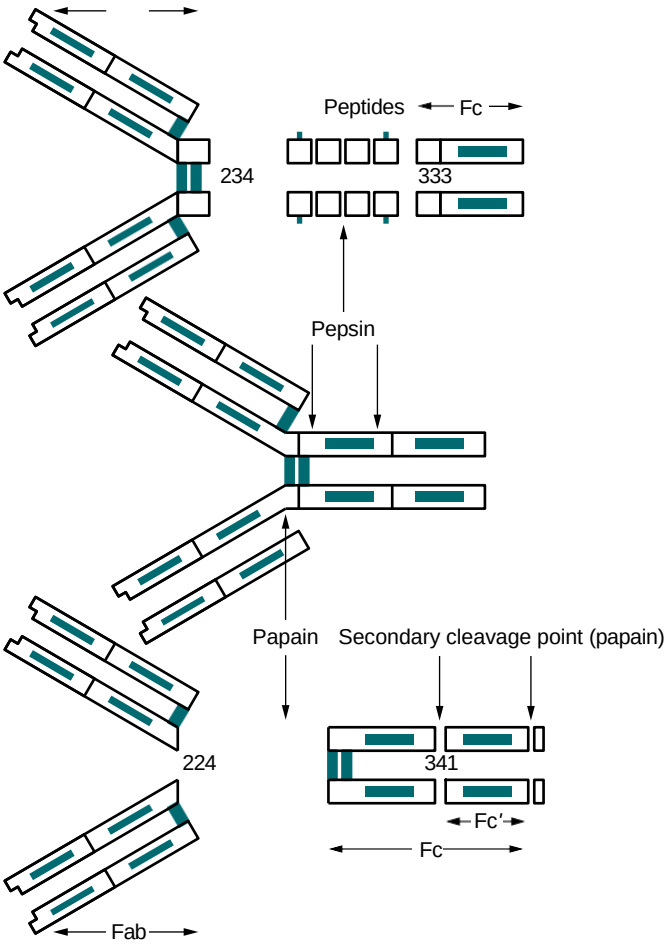


Figure 2-7 Enzymatic cleavage of human IgG1. *(Adapted from Turgeon ML: Fundamentals of immunohematology, ed 2, 1995, Williams & Wilkins.)*

Structures of Other Immunoglobulins

Immunoglobulin M

The IgM molecule is structurally composed of five basic subunits. Each subunit consists of two κ or two λ light chains and two mu (μ) heavy chains. The individual monomers of IgM are linked together by disulfide bonds in a circular fashion (Fig. 2-8). A small, cysteine-rich polypeptide, the J chain, must be considered an integral part of the molecule. IgM has carbohydrate residues attached to the C_{H3} and C_{H4} domains. The site for complement activation by IgM is located on this C_{H4} region. IgM is more efficient than IgG in activities such as complement cascade activation and agglutination.

Immunoglobulin A

In humans, more than 80% of IgA occurs as a typical four-chain structure consisting of paired κ or λ chains and two heavy chains (Fig. 2-9). The basic four-chain monomer has an MW of 160,000 Da; however, in most mammals, plasma IgA occurs mainly as a dimer. In dimeric IgA, the molecules are joined by a J chain linked to the Fc regions. Secretory IgA exists mainly in the 11S dimeric form and has an MW of 385,000 Da (Fig. 2-10). This form of IgA is present in fluids and is stabilized against proteolysis when combined with another protein, the secretory component. In humans, variations in the heavy chains account for the subclasses IgA1 and IgA2.

Immunoglobulin D

The IgD molecule has an MW of 184,000 Da and consists of two κ or α light chains and two delta (δ) heavy chains (Fig. 2-11). It has no interchain disulfide bonds between its heavy chains and an exposed hinge region.

Immunoglobulin E

The IgE molecule is composed of paired κ or α light chains and two epsilon (ε) heavy chains (Fig. 2-12). It is unique in that its Fc region binds strongly to a receptor on mast cells and basophils and, together with antigen, mediates the release of histamines and heparin from these cells.

IMMUNOGLOBULIN VARIANTS

An antigenic determinant is the specific chemical determinant group or molecular configuration against which the immune response is directed. Because they are proteins, immunoglobulins themselves can function as effective antigens when used to immunize mammals of a different species. When the resulting antiimmunoglobulins or antiglobulins are analyzed, three principal categories of antigenic determinants can be recognized—isotype, allotype, and idiotype (Fig. 2-13; Table 2-3).

Isotype Determinants

The isotypic class of antigenic determinants is the dominant type found on the immunoglobulins of all animals of a species.

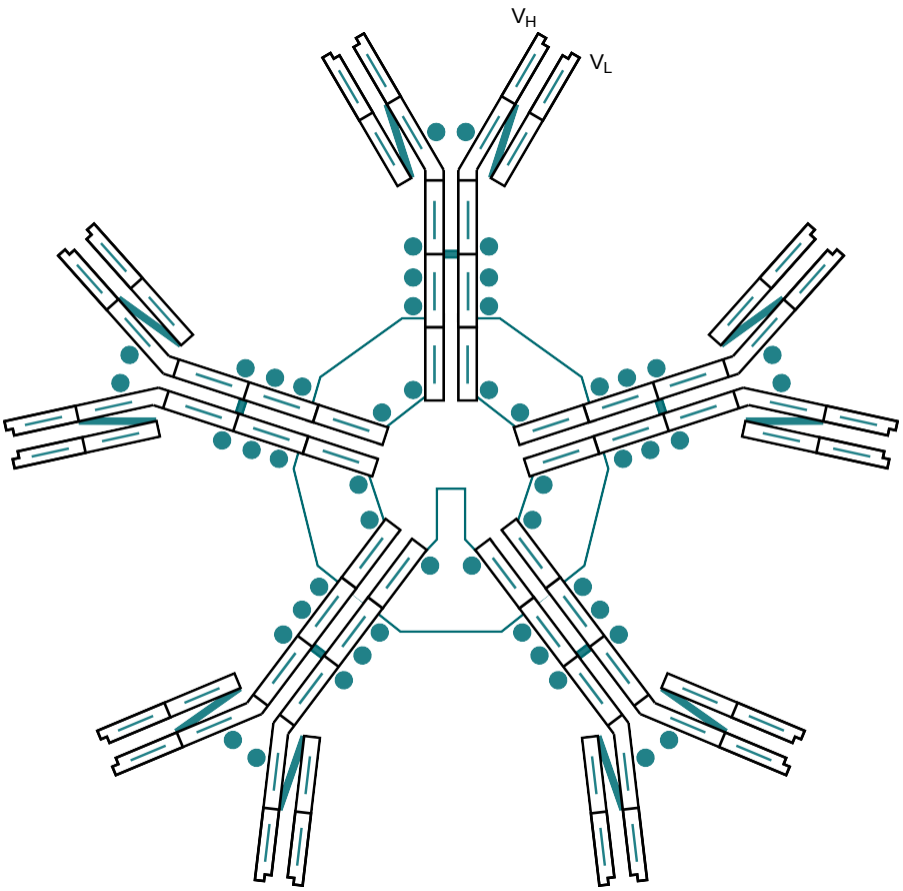


Figure 2-8 Pentameric polypeptide chain structure of human IgM. *(Adapted from Turgeon ML: Fundamentals of immunohematology, ed 2, Baltimore, 1995, Williams & Wilkins.)*

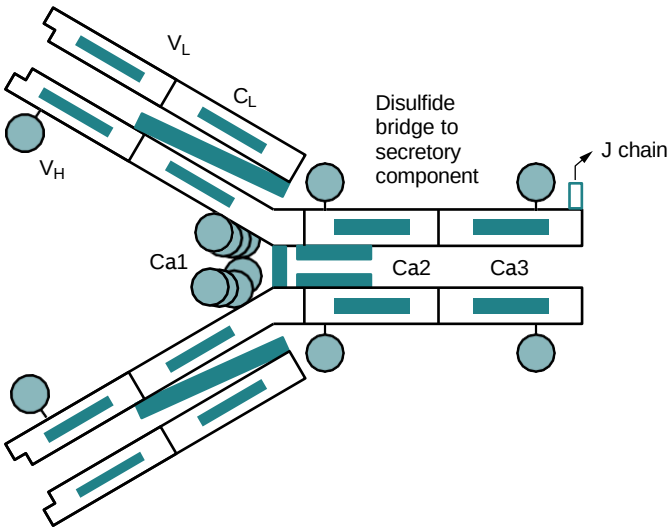


Figure 2-9 Molecule of IgA. *(From Turgeon ML: Fundamentals of Immunohematology, ed 2, Baltimore, 1995, Williams & Wilkins.)*

The heavy-chain, constant region structures associated with the different classes and subclasses are termed *isotypic variants*. Genes for isotypic variants are present in all healthy members of a species. Determinants in this category include those specific for each Ig class, such as gamma (γ) for IgG, mu (μ) for IgM, and alpha (α) for IgA, as well as the subclass-specific determinants κ and λ.

Allotype Determinants

The second principal group of determinants is found on the immunoglobulins of some, but not all, animals of a species. Antibodies to these allotypes **(alloantibodies)** may be produced by injecting the immunoglobulins of one animal into another member of the same species. The allotypic determinants are genetically determined variations representing the presence of allelic genes at a single locus within a species. Typical allotypes in humans are the Gm specificities on IgG (Gm is a marker on IgG). In humans, five sets of allotypic markers have been found—Gm, Km, Mm, Am, and Hv.

Idiotype Determinants

A result of the unique structures on light and heavy chains, individual determinants characteristic of each antibody are called **idiotypes.** The idiotypic determinants are located in the variable part of the antibody associated with the hypervariable regions that form the antigen-combining site.

ANTIBODY SYNTHESIS

When an antigen is initially encountered, the cells of the immune system recognize the antigen as nonself and elicit an immune response or become tolerant of it, depending on the circumstances. An immune reaction can take the form of cell-mediated immunity (immunity dependent on T cells and macrophages) or may involve the production of

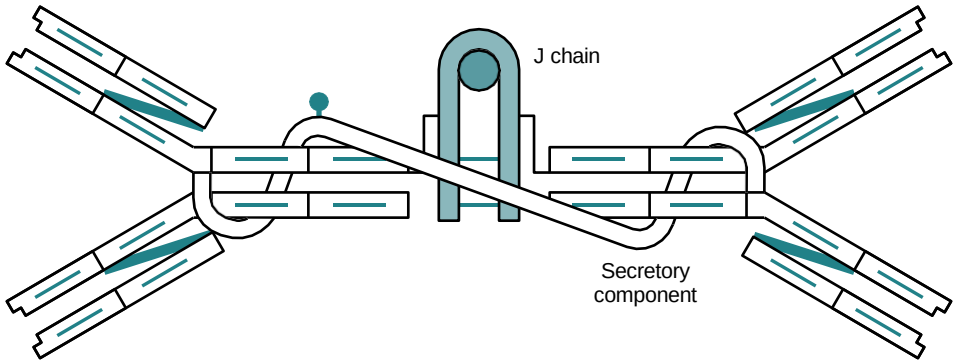


Figure 2-10 Molecule of secretory IgA. *(From Turgeon ML: Fundamentals of Immunohematology, ed 2, Baltimore, 1995, Williams & Wilkins.)*

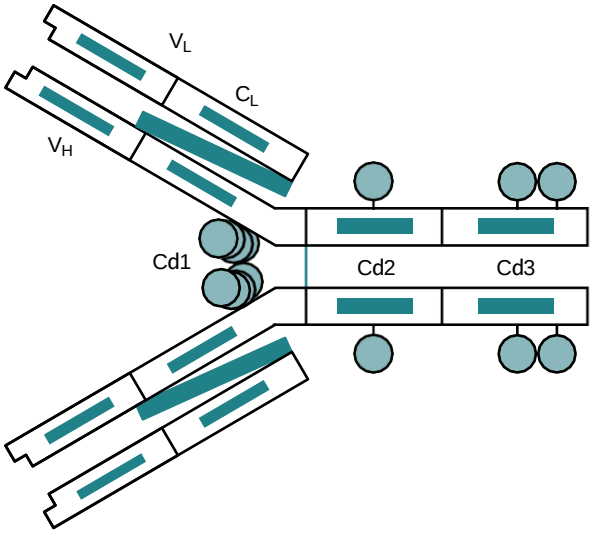


Figure 2-11 Molecule of IgD. *(From Turgeon ML: Fundamentals of Immunohematology, ed 2, Baltimore, 1995, Williams & Wilkins.)*

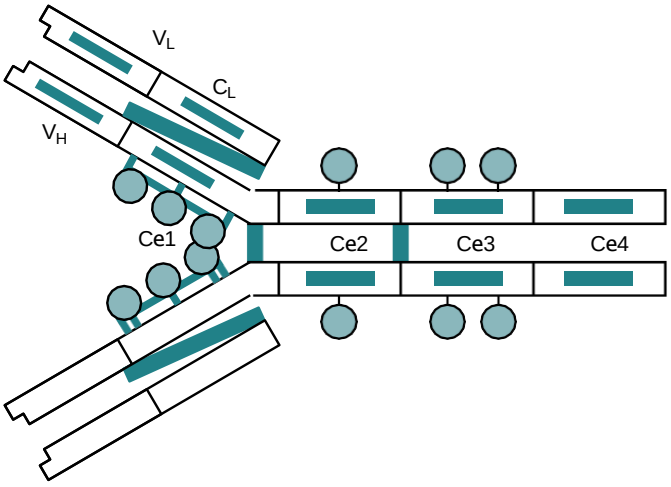


Figure 2-12 Molecule of IgE. *(From Turgeon ML: Fundamentals of Immunohematology, ed 2, Baltimore, 1995, Williams & Wilkins.)*

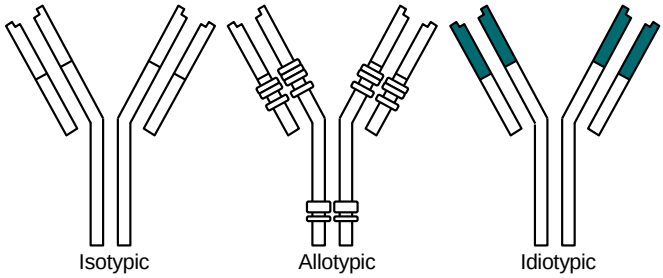


Figure 2-13 Variants of antibodies—antigenic determinants. *(Adapted from Turgeon ML: Fundamentals of immunohematology, ed 2, Baltimore, 1995, Williams & Wilkins.)*

Table 2-3 Immunoglobulin Variants

Variant	Distribution	Location	Examples
Isotype	All variants in normal persons	C_H	IgM, IgE
		C_H	IgA1, IgA2
		C_L	Kappa subtype
		C_L	Lambda subtype
Allotype	Genetically controlled alternate forms; not present in all individuals	Mainly C_H/C_L Sometimes V_H/V_2	Gm groups in humans
Idiotype	Individually specific to each immunoglobulin molecule	Variable regions	Probably one or more hypervariable regions forming the antigen-combining site

C, Constant regent; *Gm*, marker on IgG; *H*, heavy chain; *L*, light chain; *V*, variable region.

antibodies (B lymphocytes and plasma cells) directed against the antigen.

Production of antibodies is induced when the host's lymphocytes come into contact with a foreign antigenic substance that binds to its receptor. This triggers activation and proliferation, or **clonal selection.** Clonal expansion of lymphocytes in response to infection is necessary for an effective immune response (Fig. 2-14). However, it requires 3 to 5 days for a sufficient number of clones to be produced and to differentiate into antibody-producing cells. This allows time for most pathogens to damage host tissues and cells.

Whether a cell-mediated response or an antibody response takes place depends on how the antigen is presented to the lymphocytes; many immune reactions display both types of responses. The antigenicity of a foreign substance is also related to the route of entry. Intravenous and intraperitoneal routes are stronger stimuli than subcutaneous and intramuscular routes.

Subsequent exposure to the same antigen produces a memory response, or **anamnestic response,** and reflects the outcome of the initial challenge. In the case of antibody production, the quantity of IgM-IgG varies.

Primary Antibody Response

Although the duration and levels of antibody (titer) depend on the characteristics of the antigen and the individual, an IgM antibody response proceeds in the following four phases after a foreign antigen challenge (see Fig. 2-14):

1. Lag phase—no antibody is detectable.
2. Log phase—the antibody titer increases logarithmically.
3. Plateau phase—the antibody titer stabilizes.
4. Decline phase—the antibody is catabolized.

Secondary (Anamnestic) Response

Subsequent exposure to the same antigenic stimulus produces an antibody response that exhibits the same four phases as the primary response (see Fig. 2-14). Repeated exposure to an antigen can occur many years after the initial exposure, but clones of memory cells will be stimulated to proliferate, with subsequent production of antibody by the individual. An anamnestic response differs from a primary response as follows:

1. Time. A secondary response has a shorter lag phase, longer plateau, and more gradual decline.
2. Type of antibody. IgM-type antibodies are the principal class formed in the primary response. Although some IgM antibody is formed in a secondary response, the IgG class is the predominant type formed.
3. Antibody titer. In a secondary response, antibody levels attain a higher titer. The plateau levels in a secondary response are typically 10-fold or greater than the plateau levels in the primary response.

An example of an anamnestic response can be observed in hemolytic disease, when an Rh-negative mother is pregnant with an Rh-positive baby (see Chapter 26). During the mother's first exposure, the Rh-positive RBCs of the fetus leak into the maternal circulation and elicit a primary response. Subsequent pregnancies with an Rh-positive fetus will elicit a secondary (anamnestic) response.

Vaccination is the application of primary and second responses. Humans can become immune to microbial antigens through artificial and natural exposure. A vaccine is designed to provide artificially acquired active immunity to a specific disease (e.g., hepatitis B). Booster vaccine (repeated antigen exposure) allows for an anamnestic response, with an increase in antibody titer and clones of memory cells (see Chapter 16).

FUNCTIONS OF ANTIBODIES

The principal function of an antibody is to bind antigen, but antibodies may also exhibit secondary effector functions and behave as antigens. The significant secondary effector functions of antibodies are complement fixation and placental transfer (Table 2-4). The activation of complement is one of most important effector mechanisms of IgG1 and IgG3 molecules (see Chapter 5). IgG2 seems to be less effective in activating complement; IgG4, IgA, IgD, and IgE are ineffective in terms of complement activation. IgG-4 related disease is a newly recognized inflammatory condition characterized by often but not always elevated serum IgG4 concentrations.

In humans, most IgG subclass molecules are capable of crossing the placental barrier; no consensus exists on whether IgG2 crosses the placenta. Passage of antibodies across the placental barrier is important in the etiology of hemolytic disease of the fetus and newborn and in conferring passive immunity to the newborn during the first few months of life.

ANTIGEN-ANTIBODY INTERACTION: SPECIFICITY AND CROSS-REACTIVITY

The ability of a particular antibody to combine with a particular antigen is referred to as its specificity. This property resides in the portion of the Fab molecule called the combining site, a cleft formed largely by the hypervariable regions of heavy and light chains. Evidence indicates that an antigen may bind to larger, or even separate, parts of the variable region. The closer the fit between this site and the antigen determinant, the stronger are the noncovalent forces (e.g., hydrophobic or electrostatic bonds) between them, and the higher is the affinity between the antigen and antibody. Binding depends on a close three-dimensional fit, allowing weak intermolecular forces to overcome the normal repulsion between molecules. When more than one combining site interacts with the same antigen, the bond has greatly increased strength.

Antigen-antibody reactions can show a high level of specificity. Specificity exists when the binding sites of antibodies directed against determinants of one antigen are not complementary to determinants of another dissimilar antigen. When some of the determinants of an antigen are shared by similar antigenic determinants on the surface of apparently unrelated molecules, a proportion of the antibodies directed against one type of antigen will also react with the other type of antigen; this is called cross-reactivity. Antibodies directed against a protein in one species may also react in a detectable manner with the homologous protein in another species.

Cross-reactivity occurs between bacteria that possess the same cell wall polysaccharides as mammalian erythrocytes. Intestinal bacteria, as well as other substances found in the environment, possess A-like or B-like antigens similar to the A and B erythrocyte antigens. If A or B antigens are foreign to an individual, production of anti-A or anti-B occurs, despite lack of previous exposure to these erythrocyte antigens. Cross-reacting antibodies of this type are termed *heterophile antibodies.*

Antibody Affinity

Affinity is the initial force of attraction that exists between a single Fab site on an antibody molecule and a single epitope or

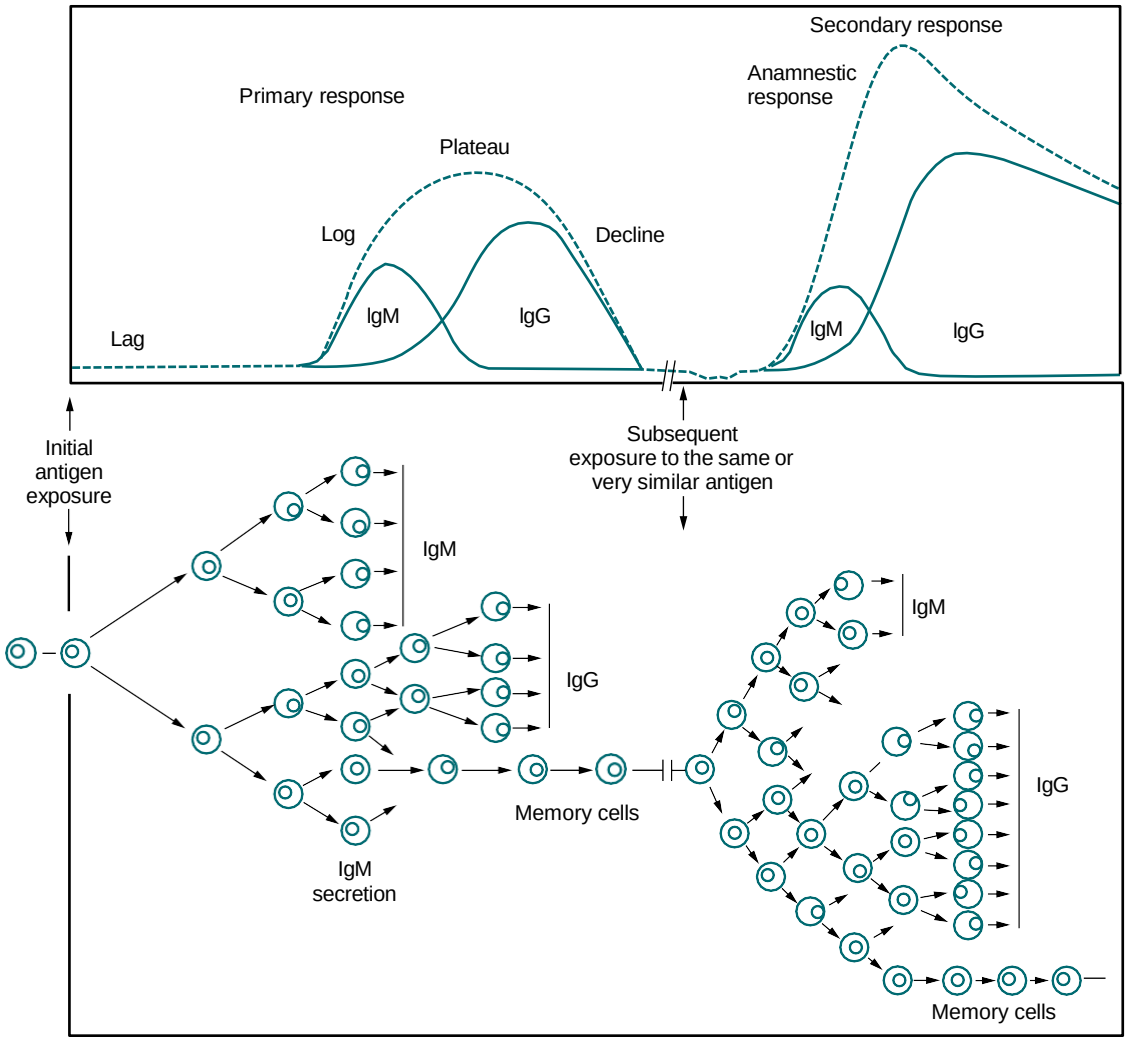


Figure 2-14 Primary and secondary antibody response. *(Adapted from Turgeon ML: Fundamentals of immunohematology, ed 2, Baltimore, 1995, Williams & Wilkins.)*

Table 2-4 Comparison of Properties of Immunoglobulins

	IgM	IgG	IgA	IgD	IgE
Complement fixation	3+	0-2+	No	No	No
Placental transfer	No	Yes	No	No	No

determinant site on the corresponding antigen. The antigen is univalent and is usually a hapten. Several types of noncovalent bonds hold an epitope and binding site close together (see later, "Type of Bonding").

Antibody Avidity

Each four-polypeptide–chain antibody unit has two antigen-binding sites, which allows them to be potentially multivalent in their reaction with an antigen. The functional combining strength of an antibody with its antigen is called **avidity,** in contrast to affinity, the binding strength between an antigenic determinant (epitope) and an antibody-combining site (Fig. 2-15). When a multivalent antigen combines with more than one of an antibody's combining sites, the strength of the bonding is significantly increased. For the antigen and antibody to dissociate, all the antigen-antibody bonds must be broken simultaneously.

Decreased avidity can result when an antigen (e.g., hapten) has only one antigenic determinant (monovalent).

Immune Complexes

The noncovalent combination of antigen with its respective specific antibody is called an **immune complex.** An immune complex may be of the small **(soluble)** or large **(precipitating)** type, depending on the nature and proportion of antigen and antibody. Under conditions of antigen or antibody excess, soluble complexes tend to predominate. If equivalent amounts of antigen and antibody are present, a precipitate may form. However, all antigen-antibody complexes will not precipitate, even at equivalence.

Antibody can react with antigen that is fixed or localized in tissues or that is released or present in the circulation. Once formed in the circulation, the immune complex is usually removed by phagocytic cells through the interaction of the Fc

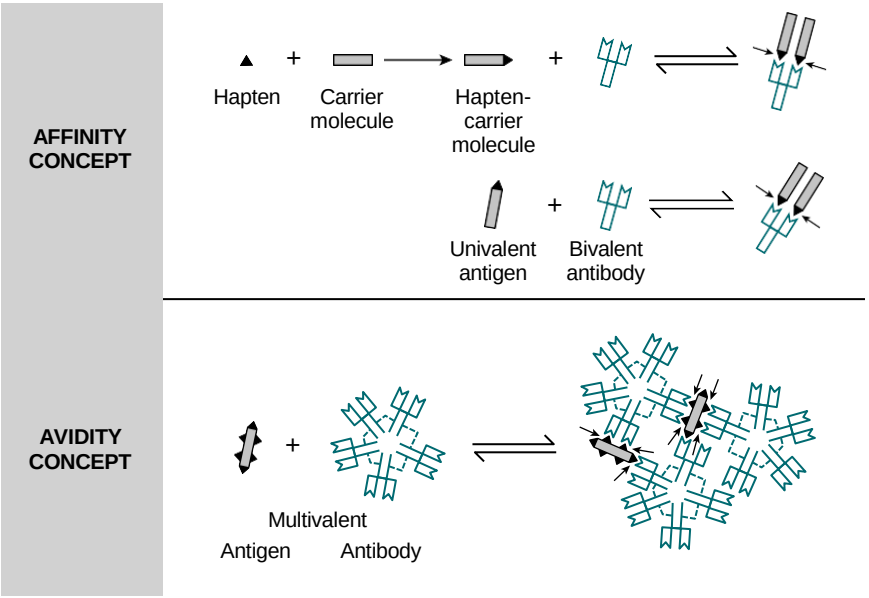


Figure 2-15 Affinity versus avidity. *(From Zane HD: Immunology: theoretical and practical concepts in laboratory medicine, Philadelphia, 2001, Saunders.)*

portion of the antibody with complement and cell surface receptors.

Under normal circumstances, this process does not lead to pathologic consequences and it may be viewed as a major host defense against the invasion of foreign antigens. It is only in unusual circumstances that the immune complex persists as a soluble complex in the circulation, escapes phagocytosis, and is deposited in endothelial or vascular structures—where it causes inflammatory damage, the principal characteristic of immune complex disease—or in organs (e.g., kidney), or inhibits useful immunity (e.g., tumors, parasites). The level of circulating immune complex is determined by the rate of formation, rate of clearance and, most importantly, nature of the complex formed. Detection of immune complexes and identification of the associated antigens are important to the clinical diagnosis of immune complex disorders.

MOLECULAR BASIS OF ANTIGEN-ANTIBODY REACTIONS

The basic Y-shaped Ig molecule is a bifunctional structure.The V regions are primarily concerned with antigen binding. When an antigenic determinant and its specific antibody combine, they interact through the chemical groups found on the surface of the antigenic determinant and on the surface of the hypervariable regions of the Ig molecule. Although the C regions do not form antigen-binding sites, the arrangement of the C regions and hinge region give the molecule segmental flexibility, which allows it to combine with separated antigenic determinants.

Types of Bonding

Bonding of an antigen to an antibody results from the formation of multiple, reversible, intermolecular attractions between an antigen and amino acids of the binding site. These forces require proximity of the interacting groups. The optimum distance separating the interacting groups varies for different types of bond; however, all these bonds act only across a very short distance and weaken rapidly as that distance increases.

The bonding of antigen to antibody is exclusively noncovalent. The attractive force of noncovalent bonds is weak compared with that of covalent bonds, but the formation of multiple noncovalent bonds produces considerable total binding energy. The strength of a single antigen-antibody bond (antibody affinity) is produced by the summation of the attractive and repulsive forces. The four types of noncovalent bonds involved in antigen-antibody reactions are hydrophobic bonds, hydrogen bonds, van der Waals forces, and electrostatic forces.

Hydrophobic Bonds

The major bonds formed between antigens and antibodies are hydrophobic. Many of the nonpolar side chains of proteins are hydrophobic. When antigen and antibody molecules come together, these side chains interact and exclude water molecules from the area of the interaction. The exclusion of water frees some of the constraints imposed by the proteins, which results in a gain in energy and forms an energetically stable complex.

Hydrogen Bonds

Hydrogen bonding results from the formation of hydrogen bridges between appropriate atoms. Major hydrogen bonds in antigen-antibody interactions are O–H–O, N–H–N, and O–H–N.

Van der Waals Forces

Van der Waals forces are nonspecific attractive forces generated by the interaction between electron clouds and hydrophobic bonds. These bonds result from minor asymmetry in the charge of an atom caused by the position of its electrons. They rely on the association of nonpolar hydrophobic groups so that contact with water molecules is minimized. Although extremely weak, van der Waals forces may become collectively important in an antigen-antibody reaction.



Figure 2-16 Goodness of fit.

Electrostatic Forces

Electrostatic forces result from the attraction of oppositely charged amino acids located on the side chains of two amino acid residues. The relative importance of electrostatic bonds is unclear.

Goodness of Fit

The strongest bonding develops when antigens and antibodies are close to each other and when the shapes of the antigenic determinants and the antigen-binding site conform to each other. This complementary matching of determinants and binding sites is referred to as goodness of fit (Fig. 2-16).

A good fit will create ample opportunities for the simultaneous formation of several noncovalent bonds and few opportunities for disruption of the bond. If a poor fit exists, repulsive forces can overpower any small forces of attraction. Variations from the ideal complementary shape will produce a decrease in the total binding energy because of increased repulsive forces and decreased attractive forces. Goodness of fit is important in determining the binding of an antibody molecule for a particular antigen.

Detection of Antigen-Antibody Reactions

In vitro tests detect the combination of antigens and antibodies. Agglutination is the process whereby particulate antigens (e.g., cells) aggregate to form larger complexes in the presence of a specific antibody. Agglutination tests are widely used in immunology to detect and measure the consequences of antigen-antibody interaction. Other tests include the following:

- Precipitation reactions combine soluble antigen with soluble antibody to produce insoluble complexes that are visible.
- Hemolysis testing involves the reaction of antigen and antibody with a cellular indicator (e.g., lysed RBCs).
- The enzyme-linked immunosorbent assay (ELISA) measures immune complexes formed in an in vitro system.

The principles of immunologic methods are discussed in Part II of this text. Detection and quantitation of immunoglobulins is important in the laboratory investigation of infectious diseases and immunologic disorders (Table 2-5).

Influence of Antibody Types on Agglutination

Immunoglobulins are relatively positively charged and, after sensitization or coating of particles, they reduce the **zeta potential,** which is the difference in electrostatic potential between the net charge at the cell membrane and the charge at the surface of shear (see Fig. 10-4). Antibodies can bridge charged particles by extending beyond the effective range of the zeta potential, which results in the erythrocytes closely approaching each other, binding, and agglutinating.

Antibodies differ in their ability to agglutinate. IgM-type antibodies, sometimes referred to as complete antibodies, are more efficient than IgG or IgA antibodies in exhibiting in vitro agglutination when the antigen-bearing erythrocytes are suspended in physiologic saline (0.9% sodium chloride solution). Antibodies that do not exhibit visible agglutination of saline-suspended erythrocytes, even when bound to the cell's surface membrane, are considered to be nonagglutinating antibodies and have been called incomplete antibodies. Incomplete antibodies may fail to exhibit agglutination because the antigenic determinants are located deep within the surface membrane or may show restricted movement in their hinge region, causing them to be functionally monovalent.

Table 2-5 Role of Specific Immunoglobulins in Diagnostic Tests

	IgG	IgM	IgA
Agglutination	1+	3+	Negative
Complement fixation	1+	3+	1+
Time of appearance after exposure to antigen (days)	3-7	2.5	3-7
Time to reach peak titer (days)	7-21	5-14	7-21

MONOCLONAL ANTIBODIES

Monoclonal antibodies are purified antibodies cloned from a single cell. These antibodies exhibit exceptional purity and specificity and are able to recognize and bind to a specific antigen.

Discovery of the Technique

In 1975, Köhler, Milstein, and Jerne discovered how to fuse lymphocytes to produce a cell line that was both immortal and a producer of specific antibodies. These scientists were awarded the Nobel Prize in Physiology and Medicine in 1984 for developing this **hybridoma** (cell hybrid) from different lines of cultured myeloma cells (plasma cells derived from malignant tumor strains). To induce the cells to fuse, they used Sendai

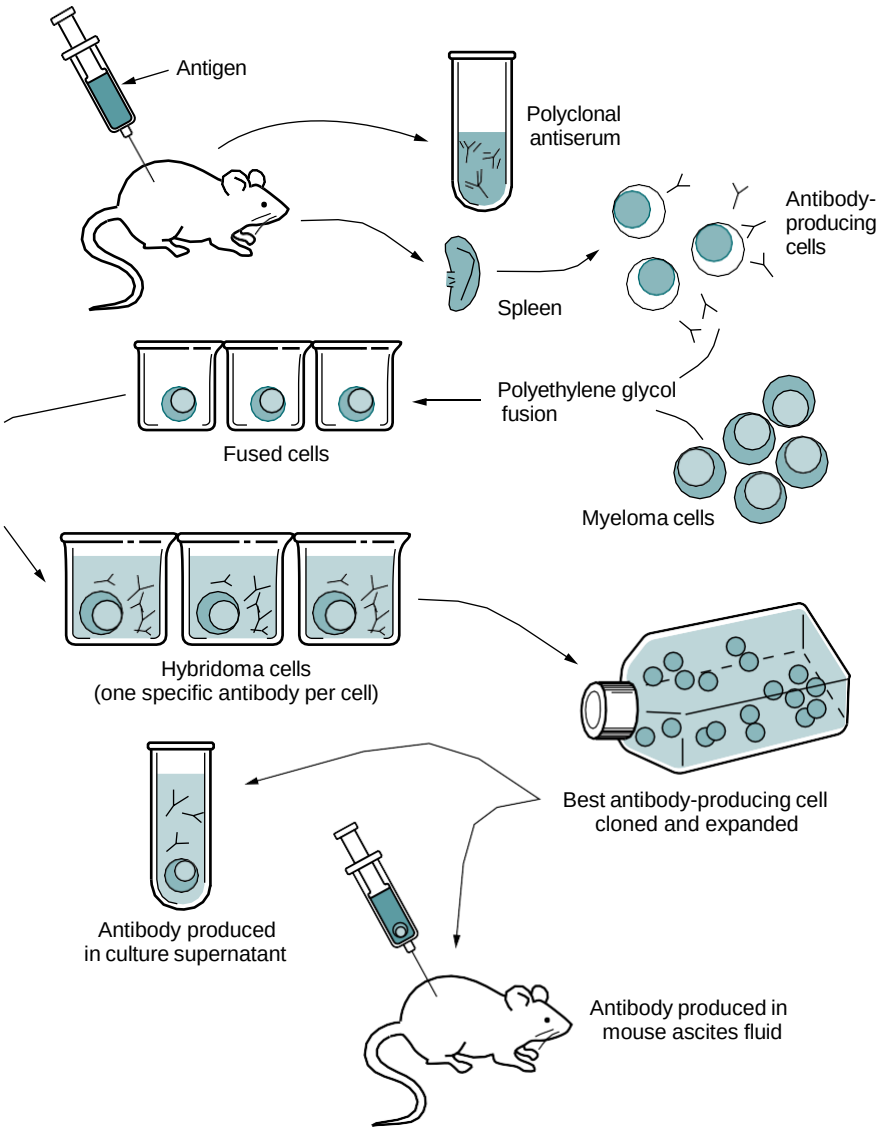


Figure 2-17 Production of monoclonal antibody (MAb). *(Adapted from Forbes BA, Sahm DF, Weissfeld AS: Bailey & Scott's diagnostic microbiology, ed 12, St Louis, 2007, Mosby.)*

virus, an influenza virus that characteristically causes cell fusion. Initially, the scientists immunized donors with sheep erythrocytes to provide a marker for the normal cells. The hybrids were tested to determine whether they still produced antibodies against the sheep erythrocytes. Köhler discovered that some of the hybrids were manufacturing large quantities of specific anti–sheep erythrocyte antibodies.

Hybrid cells secrete the antibody that is characteristic of the parent cell (e.g., anti–sheep erythrocyte antibodies). The multiplying hybrid cell culture is a hybridoma. Hybridoma cells can be cloned. The immunoglobulins derived from a single clone of cells are termed ***monoclonal antibodies (MAbs).***

Monoclonal Antibody Production

Modern methods for producing MAbs are refinements of the original technique. Basically, the hybridoma technique enables scientists to inoculate crude antigen mixtures into mice and then select clones producing specific antibodies against a single cell surface antigen (Fig. 2-17). The process of producing MAbs takes 3 to 6 months.

Mice are immunized with a specific antigen; several doses are given to ensure a vigorous immune response. After 2 to 4 days, spleen cells are mixed with cultured mouse myeloma cells. Myeloma parent cells that lack the enzyme, hypoxanthine phosphoribosyl transferase, are selected. Mouse myeloma cell lines usually do not secrete immunoglobulins, thus simplifying the purification process.

Polyethylene glycol (PEG) rather than Sendai virus is added to the cell mixture to promote cell membrane fusion. Only 1 in 200,000 spleen cells actually forms a viable hybrid with a myeloma cell. Normal spleen cells do not survive in culture. The fused cell mixture is placed in a medium containing hypoxanthine, aminopterin, and thymidine (HAT medium). Aminopterin is a drug that prevents myeloma cells from making their own purines and pyrimidines; they cannot use hypoxanthine from the medium, so they die.

Hybrids resulting from the fusion of spleen cells and myeloma cells contain transferase provided by the normal spleen cells. Consequently, the hybridoma cells are able to use the hypoxanthine and thymidine in the culture medium and

survive. They divide rapidly in HAT medium, doubling in number every 24 to 48 hours. About 300 to 500 hybrids can be generated from the cells of a single mouse spleen, although not all will be making the desired antibodies. After the hybridomas have been growing for 2 to 4 weeks, the supernatant is tested for specific antibody using methods such as ELISA. Clones that produce the desired antibody are grown in mass culture and recloned to eliminate non–antibody-producing cells.

Antibody-producing clones lose their ability to synthesize or secrete antibody after being cultured for several months. Hybridoma cells usually are frozen and stored in small aliquots. The cells may then be grown in mass culture or injected intraperitoneally into mice. Because hybridomas are tumor cells, they grow rapidly and induce the effusion of large quantities of fluid into the peritoneal cavity. This ascites fluid is rich in MAbs and can be easily harvested.

Uses of Monoclonal Antibodies

The greatest impact of MAbs in immunology has been on the analysis of cell membrane antigens. Because they have a single specificity rather than the range of antibody molecules present in the serum, MAbs have multiple clinical applications, including the following:

- Identifying and quantifying hormones
- Typing tissue and blood
- Identifying infectious agents
- Identifying clusters of differentiation for the classification of leukemias and lymphomas and follow-up therapy
- Identifying tumor antigens and autoantibodies
- Delivering immunotherapy (see Chapter 33)

CASE STUDY

History and Physical Examination

A 38-year-old white woman presented to the emergency department of her local hospital with increasing difficulty in breathing. She also reported that she had experienced chronic diarrhea for the past 18 months.

Her physical examination revealed a cachectic woman with bilateral rales and splenomegaly. After a chest x-ray film confirmed the presence of pneumonia and bronchiectasis, the patient was admitted to the hospital.

The patient's condition worsened. Her respiratory insufficiency increased and she developed renal failure and disseminated intravascular coagulation (DIC). She was subsequently transferred to a tertiary care medical center.

Medical History

The patient had a childhood history of multiple episodes of bronchitis and middle ear infections (otitis media). In her late 20s, she developed sinusitis, frequent diarrhea, and a chronic productive cough. She had two bouts of pneumonia, one of which required hospitalization. One year before the current episode, the patient developed extreme difficulty in breathing when exercising. During the past year she lost almost 30 pounds and became so weak that she could no longer lead a normal life.

Family History

She had no family history of frequent infections, immunodeficiency, or autoimmune disorders.

Laboratory Data

On admission to the tertiary medical center, a blood count, serum protein, serum protein electrophoresis, immunoglobulin electrophoresis, stool culture, and ova and parasite examination were performed.

Assay	Patient's Results	Reference Range
Complete Blood Count		
Hemoglobin	9.8 g/dL	11.5-13.5 g/dL
Hematocrit	24%	34%-42%
Total leukocyte count	9.0×10^9/L	$4.5\text{-}9.0\times10^9$/L
Polymorphonuclear leukocytes	87%	40%-60%
Lymphocytes	13%	20%-40%
Absolute lymphocytes	1.17×10^9/L	$>1.1\times10^9$/L
Other Tests		
Stool culture	Normal biota (flora)	Normal biota (flora)
Ova and parasite examination	*Giardia lamblia*	Negative for all ova and parasites
Serum total protein	5.5 g/dL	
Immunoelectrophoresis		
IgM	0.7 g/L	0.6-2.5 g/L
IgG	2.2 g/L	6.8-15.5 g/L
IgA	Undetectable	0.7-3.0 g/L
Follow-Up		
CD4+	20%	35%-55%
CD8+	26%	18%-32%
Absolute CD4+ count	0.26×10^9/L	$>0.43\times10^9$/L

The patient was found to be anergic. Tetanus, rubella, and diphtheria titers were nonprotective, despite previous immunizations.

The patient was diagnosed with common variable immunodeficiency (CVID). She was treated with IV immunoglobulin monthly. She also received metronidazole for *Giardia lamblia* intestinal infection. After 1 year of Ig therapy, the patient gained weight and returned to a normal lifestyle.

Continued

Questions

1. An immunodeficiency disorder may be suggested by:
   a. Presence of anemia
   b. History of repeated childhood infections
   c. Presence of anergy
   d. Elevated total leukocyte count
2. The most significant laboratory findings contributing to a diagnosis is:
   a. Absolute lymphocyte count
   b. Decreased CD4+ cell count
   c. Decreased immunoglobulin levels
   d. Both b and c

See Appendix A for the answers to these questions.

Critical Thinking Group Discussion Questions

1. Does the patient's medical history suggest an immunodeficiency?
2. Which laboratory findings are significant?
3. What are possible diagnoses for this patient?

See instructor site Evolve for the discussion of the answers to these questions.

ABO Blood Grouping (Forward Antigen Typing)

Principle

The ABO blood groups (A, B, AB, and O) represent the antigens expressed on the erythrocytes (red blood cells, RBCs) of each group.

Reagent typing sera contains specific antibodies to A antigen and B antigen. When an unknown patient's RBCs are mixed with known antibody A or antibody B, agglutination of the RBCs will occur if a specific antigen-antibody reaction occurs. This is called direct blood typing.

Agglutination Reactions

Anti-A	Anti-B	Blood Group
Positive	Negative	A
Negative	Positive	B
Positive	Positive	AB
Negative	Negative	O

Refer to Evolve for the procedural protocol, sources of error, and clinical notes.

Serum Protein Electrophoresis

Principle

Serum protein electrophoresis is used to separate and quantitate serum proteins based on electrophoretic mobility on cellulose acetate (see Color Plate 3 and Fig. 11-2).

Proteins are large molecules composed of amino acids. Depending on electron distributions resulting from covalent or ionic bonding of structural subgroups, proteins have different electrical charges at a given pH. Based on electrical charge, serum proteins can be fractionated into five fractions: albumin, alpha-1 (α1), alpha-2 (α2), beta (β), and gamma (γ) proteins. For the following method, the pH is 8.8. After the proteins are separated, the plate is placed in a solution of sulfosalicylic acid and Ponceau S to stain the protein bands. The intensity of the stain for each band is related to protein concentration.

Results

The fastest moving band, and normally the most prominent, is the albumin band found closest to the anodic edge of the plate. The faint band next to this is alpha-1 globulin, followed by alpha-2, beta, and gamma globulins. Prealbumin is seldom visible with this system.

Reference Values

Each laboratory should establish its own range. The following reference values are for illustrative purposes only.

Protein Fraction	Concentration (g/dL)
Albumin	3.63-4.91
Alpha-1	0.11-0.35
Alpha-2	0.65-1.17
Beta	0.74-1.26
Gamma	0.58-1.74

Clinical Interpretation

Electrophoresis is used to identify the presence or absence of aberrant proteins and to determine when different groups of proteins are increased or decreased in serum or urine. It is frequently ordered to detect and identify monoclonal proteins—excessive production of one specific immunoglobulin. Protein and immunofixation electrophoresis are ordered to help detect, diagnose, and monitor the course and treatment of conditions associated with these abnormal proteins (e.g.,multiple myeloma).

CHAPTER HIGHLIGHTS

- Foreign substances can be immunogenic if their membrane or molecular components contain structures (antigenic determinants or epitopes) recognized as foreign by the immune system. The normal immune system responds to foreignness by producing antibodies.
- Cellular antigens of importance to immunologists include MHC groups and HLAs, autoantigens, and blood group antigens. Some of these antigens (e.g., MHC) are more potent than others in provoking an immune response.
- Antigens are usually large organic molecules that are proteins or polysaccharides. Although large foreign molecules are better antigens, haptens can bind to larger carrier molecules and behave like antigens.
- Antibodies that are specific proteins are known as immunoglobulins. Many antibodies can be isolated in the

gamma globulin fraction of protein by electrophoretic separation. The primary function of an antibody in body defenses is to combine with antigen.

- Five distinct classes of immunoglobulin molecules are recognized—IgM, IgG, IgA, IgD, and IgE. Antibodies exhibit diversity among the different classes, suggesting different functions in addition to their primary function of antigen binding.
- A typical monomeric IgG molecule consists of three globular regions (two Fab regions and Fc portion) linked by a flexible hinge region.
- An antigenic determinant is the specific chemical determinant group or molecular configuration against which the immune response is directed. Because they are proteins, immunoglobulins can function as effective antigens when used to immunize mammals of a different species. When the resulting antiimmunoglobulins or antiglobulins are analyzed, three principal categories of antigenic determinants can be recognized—isotype, allotype, and idiotype.
- Production of antibodies is induced when the host's immune system comes into contact with a foreign antigenic substance and reacts to this antigenic stimulation. When an antigen is encountered initially, the cells of the immune system recognize the antigen as nonself and elicit an immune response or become tolerant of it. An immune reaction can be cell-mediated immunity (dependent on T cells and macrophages) or may involve the production of antibodies directed against the antigen.
- After a foreign antigen challenge, an IgM antibody response proceeds in four phases—lag, log, plateau, and decline. Subsequent exposure to the same antigenic stimulus produces an anamnestic (secondary) response, which exhibits the same four phases but differs from a primary response in time, type of antibody produced, and antibody titer.
- Specificity is the ability of a particular antibody to combine with one antigen instead of another.
- Affinity is the bonding strength between an antigenic determinant and antibody-combining site, whereas avidity is the strength with which a multivalent antibody binds a multivalent antigen.
- Agglutination and other tests (e.g., precipitation reactions, hemolysis testing, ELISA) are widely used in immunology to detect and measure the consequences of antigen-antibody interaction.
- Monoclonal antibodies (MAbs) are purified antibodies cloned from a single cell. MAbs bound to cell surface antigens now provide a method for classifying and identifying specific cellular membrane characteristics and leukocyte antigens.

REVIEW QUESTIONS

1. A synonym for an antigenic determinant is:
   a. Immunogen
   b. Epitope
   c. Binding site
   d. Polysaccharide

2. Genetically different individuals of the same species are referred to as:
   a. Allogenic
   b. Heterogenic
   c. Autogenic
   d. Isogenic

3. Antigenic substances can be composed of:
   a. Large polysaccharides
   b. Proteins
   c. Glycoproteins
   d. All of the above

4. Which of the following characteristics of an antigen is the least important?
   a. Foreignness
   b. Degradability
   c. Molecular weight
   d. Presence of large repeating polymers

5. The chemical composition of an antibody is:
   a. Protein
   b. Lipid
   c. Carbohydrate
   d. Any of the above

6-10. Match the following characteristics with the appropriate antibody class (use an answer only once).

6. _____ IgM
7. _____ IgG
8. _____ IgA
9. _____ IgE
10. _____ IgD
    a. Highest in plasma or serum concentration in normal individuals
    b. Shortest half-life
    c. 19S
    d. Can exist as a dimer
    e. No known subclasses

11-15. Match the following characteristics with the appropriate antibody (use an answer only once).

11. _____ IgG
12. _____ IgM
13. _____ IgA
14. _____ IgD
15. _____ IgE
    a. Predominant immunoglobulin in secretions
    b. Increased in infectious diseases, collagen disorders, and hematologic disorders
    c. Mediates some types of hypersensitivity reactions
    d. Primarily a cell membrane immunoglobulin
    e. Produced early in an immune response

16-18. Match each of the following antigenic determinant terms with its appropriate definition.

16. _____ Isotype

17. _____ Allotype

18. _____ Idiotype
    a. Found on the immunoglobulins of some, but not all, animals of a species
    b. Dominant type found on immunoglobulins of all animals of a species
    c. Individual determinants characteristic of each antibody

19-22. Arrange the sequence of events of a typical antibody response.

19. _____

20. _____

21. _____

22. _____
    a. Plateau
    b. Lag phase
    c. Log phase
    d. Decline

23. Which of the following statements is false about an anamnestic response versus a primary response?
    a. Has a shorter lag phase
    b. Has a longer plateau
    c. Antibodies decline more gradually.
    d. IgM antibodies predominate.

24. Which type of antibody is capable of placental transfer?
    a. IgM
    b. IgG
    c. IgA
    d. IgD

25-28. Match the following terms and their respective definitions.

25. _____ Specificity

26. _____ Affinity

27. _____ Avidity

28. _____ Immune complex
    a. Strength of a bond between a single antigenic determinant and an individual combining site
    b. Noncovalent combination of an antigen with its respective specific antibody
    c. Ability of an antibody to combine with one antigen instead of another
    d. Strength with which a multivalent antibody binds to a multivalent antigen

29. Which of the following type(s) of bonding is (are) involved in antigen-antibody reactions?
    a. Hydrophobic
    b. Hydrogen
    c. Van der Waals
    d. All of the above

30. Monovalent antibodies have also been referred to as:
    a. Complete antibodies
    b. Incomplete antibodies

31. Which of the following is an accurate statement about monoclonal antibodies (MAbs)?
    a. MAbs are antibodies engineered to bind to a single epitope.
    b. MAbs are purified antibodies cloned from a single cell.
    c. MAbs are used to classify and identify specific cellular membrane characteristics.
    d. All of the above are correct.

32. Antigens are characterized by all the following except that they:
    a. Are usually large organic molecules
    b. Are usually lipids
    c. Can be glycolipids or glycoproteins
    d. Are also called immunogens

33. The immunogenicity of an antigen depends greatly on:
    a. Its biochemical composition
    b. Being structurally unstable
    c. Its degree of foreignness
    d. Having a low molecular weight

34. Antibodies are also referred to as:
    a. Immunoglobulins
    b. Haptens
    c. Epitopes
    d. Gamma globulins

35-39. Match the following immunoglobulins with the appropriate description.

35. _____ IgM

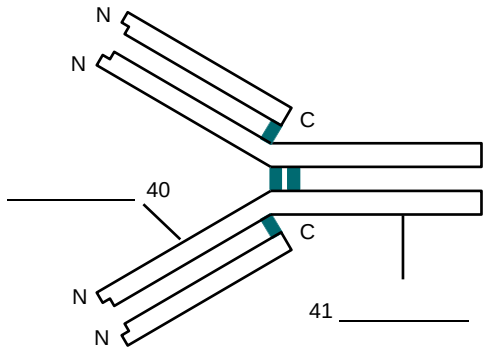
36. _____ IgG

37. _____ IgA

38. _____ IgE

39. _____ IgD
    a. Accounts for 10% of Ig pool, largely confined to the intravascular space
    b. Mediates some types of hypersensitivity
    c. Found in tears, saliva, colostrum, milk, and intestinal secretions
    d. Makes up less than 1% of total immunoglobulins
    e. Diffuses more readily into extravascular spaces, neutralizes toxins, and binds to microorganisms

40 and 41. Label the components of the basic immunoglobulin (Ig) configuration in the following figure.



(Adapted from Turgeon ML: Fundamentals of immunohematology, ed 2, Baltimore, 1995, Williams & Wilkins.)

Possible answers for question 40:

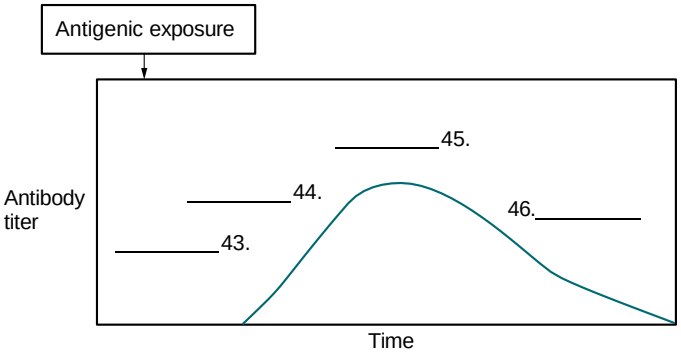
a. Fc segment
b. Fab segment
c. Hinge region
d. Disulfide bond

Possible answers for question 41:

a. Fc segment
b. Fab segment
c. Hinge region
d. Disulfide bond

42. Which of the following statements about IgM is false?

a. Composed of five basic subunits
b. More efficient in the activation of the complement cascade and agglutination than IgG
c. Predominant in an initial antibody response
d. Predominant in a secondary (anamnestic) response



(Adapted from Turgeon ML: Fundamentals of immunohematology, ed 2, Baltimore, 1995, Williams & Wilkins.)

43-46. Label the four phases of an antibody response on the following figure, choosing from the following answers:

a. Log
b. Plateau
c. Lag
d. Decline

47. In a secondary (anamnestic) response, all the following characteristics are correct *except:*

a. IgG is the predominant antibody type
b. It has a shorter lag phase
c. The antibody titer is lower
d. It has a more gradual decline in antibody response

48. Bonding of antigen to antibody exists exclusively as:

a. Hydrogen bonding
b. Van der Waals forces
c. Electrostatic forces
d. Noncovalent bonding

49. The strongest bond of antigen and antibody chiefly results from the:

a. Type of bonding
b. Goodness of fit
c. Antibody type
d. Quantity of antibody

50. Monoclonal antibodies have all the following characteristics except:

a. Purified antibodies
b. Cloned from a single cell
c. Engineered to bind to a single specific antigen
d. Frequent occurrence in nature

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CHAPTER 3

Cells and Cellular Activities of the Immune System: Granulocytes and Mononuclear Cells

Origin and Development of Blood Cells
Granulocytic Cells
- Neutrophils
- Eosinophils and Basophils

Process of Phagocytosis
- Chemotaxis
- Adherence
- Engulfment
- Digestion
- Subsequent Phagocytic Activity
- Neutrophil Extracellular Traps

Monocytes-Macrophages
- Mononuclear Phagocyte System
- Host Defense Functions

Acute Inflammation
Sepsis
Cell Surface Receptors
Disorders of Neutrophils
- Noninfectious Neutrophil-Mediated Inflammatory Disease
- Abnormal Neutrophil Function
- Congenital Neutrophil Abnormalities

Monocyte-Macrophage Disorders
- Gaucher's Disease
- Niemann-Pick Disease

Disease States Involving Leukocyte Integrins
Case Studies
- Questions
- Critical Thinking Group Discussion Questions

Procedure: Screening Test for Phagocytic Engulfment
Chapter Highlights
Review Questions
Bibliography

Learning Objectives

At the conclusion of this chapter, the reader should be able to:

- Describe the general functions of granulocytes, monocytes-macrophages, and lymphocytes and plasma cells as components of the immune system.
- Explain the process of phagocytosis.
- Describe the composition and function of neutrophil extracellular traps (NETs).
- Discuss the role of monocytes and macrophages in cellular immunity.
- Define and compare acute inflammation and sepsis.
- Briefly describe cell surface receptors.
- Name and compare the signs and symptoms of disorders of neutrophil function.
- Compare the signs and symptoms of two monocyte or macrophage disorders.
- Describe states involving the leukocyte integrins.
- Analyze case studies related to defects of neutrophils.
- Correctly answer case study related multiple choice questions.
- Be prepared to participate in a discussion of critical thinking questions.
- Describe the principal reporting of results, sources of error, clinical applications, and limitations of a phagocytic engulfment test.
- Correctly answer end of chapter review questions.

Key Terms

bacteremia
cell adhesion molecules (CAMs)
cell surface receptors
Chédiak-Higashi Syndrome
chemoattractant
chemokines
chemotaxis
chronic granulomatous disease (CGD)
complement receptor
diapedesis
endotoxin
exocytosis
extracellular matrix (ECM)
extravasation
exudate (pus)
Gaucher's disease
inflammation
interferon
leukocyte integrins
leukotrienes
ligands
macrophages
margination
neutrophil extracellular traps (NETs)
Niemann-Pick disease
opsonization
reactive oxygen species (ROS)
selectin
sepsis

The entire leukocytic cell system is designed to defend the body against disease. Each cell type has a unique function and behaves independently and, in many cases, in cooperation with other cell types. Leukocytes can be functionally divided into the general categories of granulocyte, monocyte-macrophage, and lymphocyte–plasma cell. The primary phagocytic cells are the polymorphonuclear neutrophil (PMN) leukocytes and the mononuclear monocytes-macrophages. The response of the body to pathogens involves cross-talk among many immune cells, including **macrophages,** dendritic cells, and CD4 T cells (Fig. 3-1). The lymphocytes participate in body defenses primarily through the recognition of foreign antigens and production of antibody. Plasma cells are antibody-synthesizing cells.

ORIGIN AND DEVELOPMENT OF BLOOD CELLS

Embryonic blood cells, excluding the lymphocyte type of white blood cell (WBC), originate from the mesenchymal tissue that arises from the embryonic germ layer, the mesoderm. The sites of blood cell development, hematopoiesis, follow a definite sequence in the embryo and fetus:

1. The first blood cells are primitive red blood cells (RBCs; erythroblasts) formed in the islets of the yolk sac during the first 2 to 8 weeks of life.
2. Gradually, the liver and spleen replace the yolk sac as the sites of blood cell development. By the second month of gestation, the liver becomes the major site of hematopoiesis, and granular types of leukocytes have made their initial appearance. The liver and spleen predominate from about 2 to 5 months of fetal life.
3. In the fourth month of gestation, bone marrow begins to produce blood cells. After the fifth fetal month, bone marrow begins to assume its ultimate role as the primary site of hematopoiesis.

The cellular elements of the blood are produced from a common, multipotential, hematopoietic (blood-producing) cell, the stem cell. After stem cell differentiation, blast cells arise for each of the major categories of cell types—erythrocytes, megakaryocytes, granulocytes, monocytes-macrophages, lymphocytes, and plasma cells. Subsequent maturation of these cells will produce the major cellular elements of the circulating blood, the erythrocytes (RBCs), thrombocytes, and specific types of leukocytes (WBCs). In normal peripheral or circulating blood, the following types of leukocytes can be found, in order of frequency: neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

GRANULOCYTIC CELLS

Granulocytic leukocytes can be further subdivided on the basis of morphology into neutrophils, eosinophils, and basophils. Each of these begins as a multipotential stem cell in the bone marrow.

Neutrophils

Neutrophilic leukocytes, particularly the polymorphonuclear (PMN) type (see Color Plate 4), provide an effective host defense against bacterial and fungal infections.The antimicrobial function of PMNs is essential in the innate immune response. Although the monocytes-macrophages and other granulocytes are also phagocytic cells, the PMN is the principal leukocyte

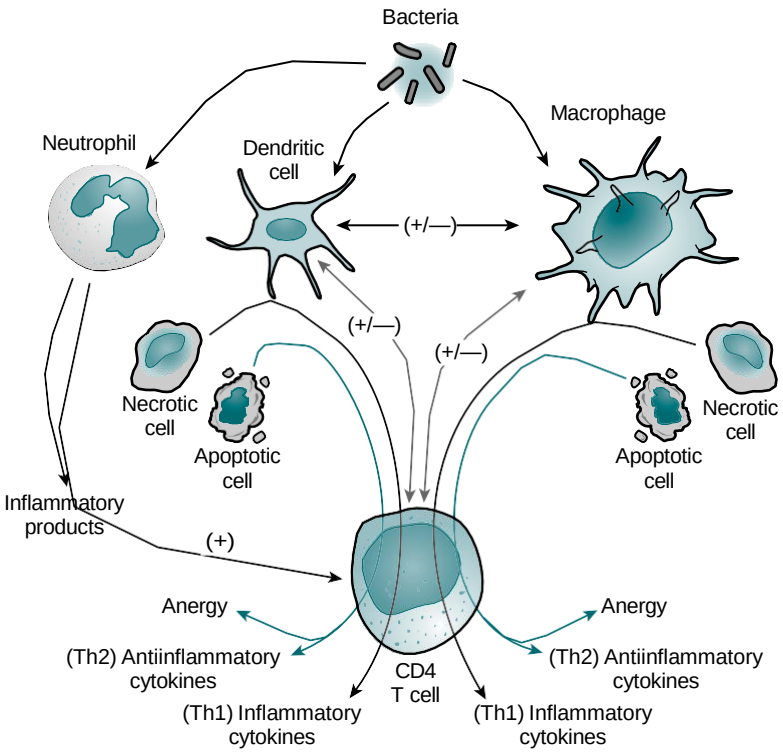


Figure 3-1 Response of pathogens, involving cross-talk among many immune cells, including macrophages, dendritic cells, and CD4 T cells. Macrophages and dendritic cells are activated by the ingestion of bacteria and by stimulation through cytokines (e.g., IFN-γ) secreted by CD4 T cells. Alternatively, CD4 T cells that have an antiinflammatory profile (type 2 helper T cell [Th2]) secrete IL-10, which suppresses macrophage activation. CD4 T cells become activated by stimulation through macrophages or dendritic cells. For example, macrophages and dendritic cells secrete IL-12, which activates CD4 T cells to secrete inflammatory (type 1 helper T cell [Th1]) cytokines. Depending on numerous factors (e.g., type of organism and site of infection), macrophages and dendritic cells respond by inducing inflammatory or antiinflammatory cytokines or causing a global reduction in cytokine production (anergy). Macrophages or dendritic cells that have previously ingested necrotic cells induce an inflammatory cytokine profile (Th1). Ingestion of apoptotic cells can induce an antiinflammatory cytokine profile or anergy. A plus sign indicates upregulation and a minus sign indicates downregulation; in cases in which both a plus sign and a minus sign appear, upregulation or downregulation may occur, depending on a variety of factors. *(Adapted from Hotchkiss RS, Karl IE: The pathophysiology and treatment of sepsis. N Engl J Med 348:138–150, 2003.)*

associated with phagocytosis and a localized inflammatory response. The formation of an inflammatory **exudate (pus),** which develops rapidly in an inflammatory response, is composed primarily of neutrophils and monocytes.

PMNs can prolong **inflammation** by the release of soluble substances, such as cytokines and chemokines. The role of neutrophils in influencing the adaptive immune response is believed to include shuttling pathogens to draining lymph nodes, antigen presentation, and modulation of T helper types 1 and 2 responses. Functionality of neutrophils is no longer considered as limited as it once was because new research has discovered that PMNs have a 5.4 day lifespan.

Mature neutrophils are found in two evenly divided pools, the circulating and marginating pools. The marginating granulocytes adhere to the vascular endothelium. In the peripheral blood, these cells are only in transit to their potential sites of action in the tissues. Movement of granulocytes from the circulating pool to the peripheral tissues occurs by a process called **diapedesis** (movement through the vessel wall). Once in the peripheral tissues, the neutrophils are able to carry out their function of phagocytosis.

The granules of segmented neutrophils contain various antibacterial substances (Table 3-1). During the phagocytic process, the powerful antimicrobial enzymes that are released also disrupt the integrity of the cell itself. Neutrophils are also steadily lost to the respiratory, gastrointestinal (GI), and urinary systems, where they participate in generalized phagocytic activities. An alternate route for the removal of neutrophils from the circulation is phagocytosis by cells of the mononuclear phagocyte system.

Table 3-1 Function and Types of Granules in Neutrophils

Function	Azurophilic (Primary) Granules	Specific (Secondary) Granules
Microbicidal	Myeloperoxidase	Cytochrome b558 and other respiratory burst components
	Lysozyme	Lysozyme
	Elastase	Lactoferrin
	Defensins	
	Cathepsin G	
	Proteinase-3	
	Bacterial permeability-increasing protein (BPI)	
Cell migration		Collagenase
		CD11b-CD18 (CR-3)
		N-formulated peptides (e.g., *N*-formyl-methionyl-leucylphenylalanine receptor [FMLP-R])

Adapted from Peakman M, Vergani D: Basic and clinical immunology, ed 2, Edinburgh, 2009, Churchill Livingstone, p 24.

Eosinophils and Basophils

Although capable of participating in phagocytosis, eosinophils and basophils possess less phagocytic activity. The ineffectiveness of these cells results from the small number of cells in the circulating blood and lack of powerful digestive enzymes. Both eosinophils and basophils, however, are functionally important in body defense.

Eosinophils

The eosinophil (see Color Plate 5) is considered to be a homeostatic regulator of inflammation. Functionally, this means that the eosinophil attempts to suppress an inflammatory reaction to prevent the excessive spread of the inflammation. The eosinophil may also play a role in the host defense mechanism because of its ability to kill certain parasites.

A functional property related to the membrane receptors of the eosinophil is the cell's ability to interact with the larval stages of some helminth parasites and damage them through oxidative mechanisms. Certain proteins released from eosinophilic granules damage antibody-coated *Schistosoma* parasites and may account for damage to endothelial cells in hypereosinophilic syndromes.

Basophils

Basophils (see Color Plate 6) have high concentrations of heparin and histamine in their granules, which play an important role in acute, systemic, hypersensitivity reactions (see Chapter 26). Degranulation occurs when an antigen such as pollen binds to two adjacent immunoglobulin E (IgE) antibody molecules located on the surface of mast cells. The events resulting from the release of the contents of these basophilic granules include increased vascular permeability, smooth muscle spasm, and vasodilation. If severe, this reaction can result in anaphylactic shock.

A class of compounds known as **leukotrienes** mediates the inflammatory functions of leukocytes. The observed systemic reactions related to leukotrienes were previously attributed to the slow-reacting substance of anaphylaxis.

PROCESS OF PHAGOCYTOSIS

Phagocytosis can be divided into six stages—chemotaxis, adherence, engulfment, phagosome formation, fusion, and digestion and destruction (Fig. 3-2). The physical occurrence of damage to tissues, by trauma or microbial multiplication, releases substances such as activated complement components and products of infection to initiate phagocytosis.

Chemotaxis

Various phagocytic cells continually circulate throughout the blood, lymph, GI system, and respiratory tract. When trauma occurs, the neutrophils arrive at the site of injury and can be

found in the initial exudate in less than 1 hour. Monocytes are slower in moving to the inflammatory site. Macrophages resident in the tissues of the body are already in place to deal with an intruding agent. Additional macrophages from the bone marrow and other tissues can be released in severe infections.

Recruitment of PMNs is an essential prerequisite in innate immune defense. Recruitment of PMNs consists of a cascade of events that allows for the capture, adhesion, and **extravasation** of the leukocyte. Activities such as rolling binding and diapedesis have been well characterized but receptor-mediated processes, mechanisms attenuating the electrostatic repulsion between the negatively charged glycocalyx of leukocytes and endothelium, are poorly understood. Research has demonstrated that myeloperoxidase (MPO), a PMN-derived heme protein, facilitates PMN recruitment becaue of its positive surface charge.

Neutrophils have been shown to activate complement when stimulated by cytokines or coagulation-derived factors. Neutrophils activate the alternative complement pathway and release C5 fragments, which further amplify neutrophil proinflammatory responses. This mechanism may be relevant to complement involvement in neutrophil-mediated diseases.

Segmented neutrophils are able to gather quickly at the site of injury because they are actively motile. The marginating pool of neutrophils, adhering to the endothelial lining of nearby blood vessels, migrates through the vessel wall to the interstitial tissues. Mediators produced by microorganisms and by cells participating in the inflammatory process include interleukin-1 (IL-1), which is released by macrophages in response to infection or tissue injury. Another is histamine, released by circulating basophils, tissue mast cells, and blood platelets. Mediators cause capillary and venular dilation.

Cells are guided to the site of injury by **chemoattractant** substances. This event is termed ***chemotaxis.*** A chemotactic response is defined as a change in the direction of movement of a motile cell in response to a concentration gradient of a specific chemical, chemotaxin. Chemotaxins can induce a positive movement toward and a negative movement away from a chemotactic response. Antigens function as chemoattractants; when antigenic material is present in the body, phagocytes are attracted to its source by moving up its concentration gradient.

Phagocytes detect antigens using various cell surface receptors. The speed of phagocytosis can be greatly increased by recruiting the following two attachment devices present on the surface of phagocytic cells:

- Fc receptor—binds the Fc portion of antibody molecules, chiefly immunoglobulin G (IgG). The IgG attaches to the organism through its Fab site.
- **Complement receptor**—the third component of complement, C3, also binds to organisms and then attaches to the complement receptor.

This coating of the organisms by molecules that speed up phagocytosis is termed ***opsonization;*** the Fc portions of antibody and C3 are called opsonins. The steps in opsonization are as follows:

1. Antibody attached to the surface of a bacterium minimally binds the Fc phagocyte receptor.
2. Complement C3b is attached to the surface of the bacterium and binds loosely to the phagocyte C3b receptor.
3. Both antibody and C3b are attached to the surface of the bacterium and bound tightly to the phagocyte, allowing greater opportunity for the phagocyte to engulf the bacterium.

Necrotic cells release an independent chemoattractant of necrotaxis signal, which directs PMN migration beyond the intravascular chemokine gradient. This intravascular danger sensing and recruitment mechanisms have evolved to limit the collateral damage during a response to sterile injury. In this process, PMNs are allowed to migrate intravascularly as they

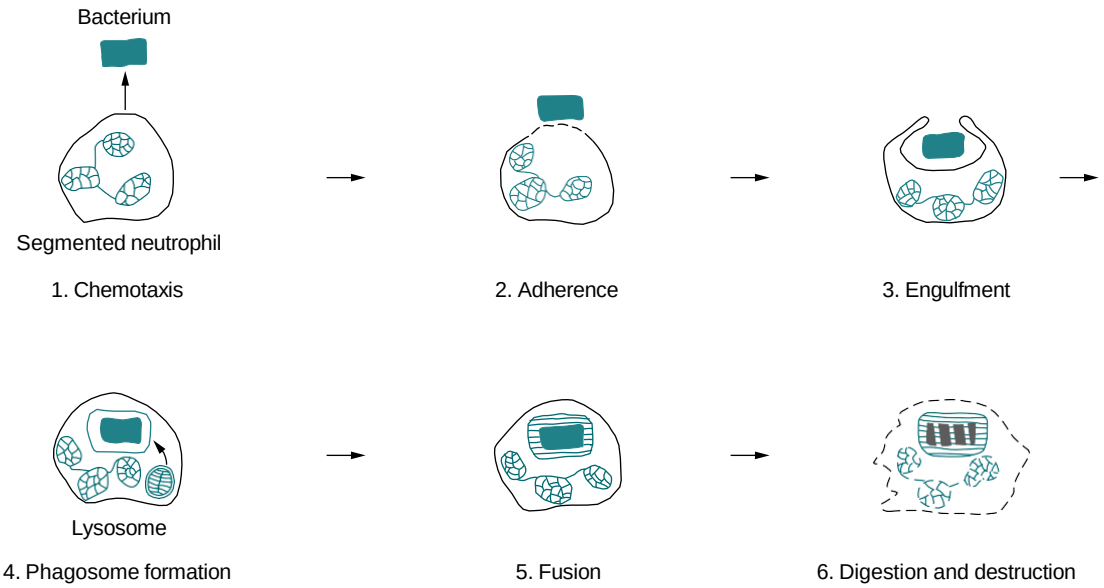


Figure 3-2 Process of phagocytosis. *(Adapted from Turgeon ML: Clinical hematology: theory and procedures, ed 5, Philadelphia, 2012, Lippincott-Williams & Wilkins.)*

navigate through healthy tissue to sites of injury. Necrotaxis signals promote localization of neutrophils directly into existing areas of injury to focus the innate immune response on damaged areas and away from healthy tissue, which provides an additional safeguard against collateral damage during sterile inflammatory responses. The innate immune system can clean up the dead by killing the living.

Adherence

The leukocyte adhesion cascade is a sequence of adhesion and activation events that ends with the cell exerting its effects on the inflamed site (see later, "Acute Inflammation"). At least five steps appear to be necessary for effective leukocyte recruitment to the site of injury—capture, rolling, slow rolling, firm adhesion, and transmigration.

The process known as capture (tethering) represents the first contact of a leukocyte with the activated endothelium. Capture occurs after **margination,** which allows phagocytes to move in a position close to the endothelium. P-selectin on endothelial cells is the primary adhesion molecule for capture and the initiation of rolling. Functional E-selectin **ligands** include CD44. In addition, many studies have suggested that L-selectin also has an important role in capture. Other **cell adhesion molecules (CAMs)** have been implicated in capture (e.g., PECAM-1, ICAM-1, VE-cadherin, LFA-1 [CD11a/CD18], IAP [CD47], VLA-4 [4β_1–integrin]), although their level of actual involvement varies.

The inflammatory response begins with a release of inflammatory chemicals into the extracellular fluid. Sources of these inflammatory mediators, the most important of which are histamine, prostaglandins, and cytokines, are injured tissue cells, lymphocytes, mast cells, and blood proteins. The presence of these chemicals promotes the reactions to inflammation (redness, heat, swelling, pain).

The transit time through the microcirculation and, more specifically, the contact time during which the leukocyte is close to the endothelium, appears to be a key parameter in determining the success of the recruitment process, as reflected in firm adhesion.

Engulfment

On reaching the site of infection, phagocytes engulf and destroy the foreign matter (Fig. 3-3). Eosinophils can also undergo this process, except that they kill parasites. After the phagocytic cells have arrived at the site of injury, the bacteria can be engulfed through active membrane invagination. Pseudopodia are extended around the pathogen, pulled by interactions between the Fc receptors and Fc antibody portions on the opsonized bacterium. Pseudopodia meet and fuse, thereby internalizing the bacterium and enclosing it in a phagocytic vacuole, or phagosome.

The principal factor in determining whether phagocytosis can occur is the physical nature of the surface of the bacteria and phagocytic cell. The bacteria must be more hydrophobic than the phagocyte. Some bacteria, such as *Diplococcus pneumoniae,* possess a hydrophilic capsule and are not normally phagocytized. Most nonpathogenic bacteria are easily phagocytized because they are very hydrophobic. The presence of certain soluble factors such as complement, a plasma protein, coupled with antibodies and chemicals such as acetylcholine enhance the phagocytic process. Enhancement of phagocytosis through opsonization can speed up the ingestion of particles. If the surface tensions are conducive to engulfment, the phagocytic cell membrane invaginates. This invagination leads to the formation of an isolated vacuole (phagosome) within the cell.

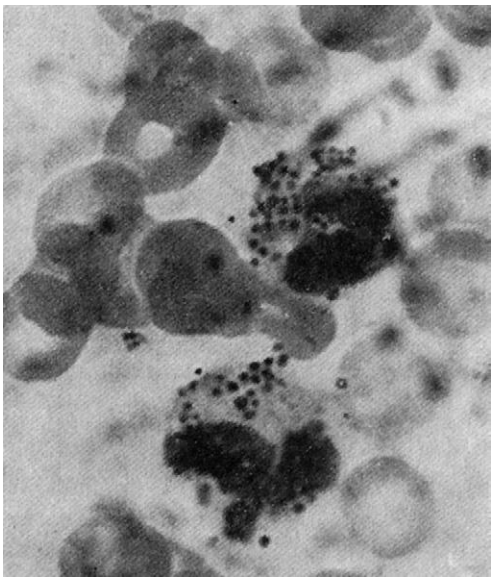


Figure 3-3 Two phagocytic cells have engulfed numerous *Staphylococcus aureus* cells. *(From Barrett JT: Textbook of immunology, ed 5, St Louis, 1988, Mosby.)*

Digestion

Digestion follows the ingestion of particles, with the required energy primarily provided by anaerobic glycolysis. Granules in the phagocyte cytosol then migrate to and fuse with the phagosome to form the phagolysosome. These granules contain degradatory enzymes of the following three types:

1. Primary, or azurophilic, granules containing enzymes (e.g., lysozyme, myeloperoxidase)
2. Secondary, or specific, granules containing substances such as lactoferrin
3. Tertiary granules containing substances such as caspases

Degranulation of the neutrophil releases antibacterial substances (e.g., lactoferrin, lysozyme, defensin) from the granules; released enzymes promote bactericidal activity by increasing membrane permeability. Elastase, one of several substances that can damage host tissues, is also released. The myeloperoxidase granules are responsible for the action of the oxygen-dependent, myeloperoxidase-mediated system. Hydrogen peroxide (H_2O_2) and an oxidizable cofactor serve as major factors in the actual killing of bacteria within the vacuole. Other oxygen-independent systems, such as alterations in pH, lysozymes, lactoferrin, and the granular cationic proteins, also participate in the bactericidal process. Monocytes are particularly effective as phagocytic cells because of

the large amounts of lipase in their cytoplasm. Lipase is able to attack bacteria with a lipid capsule, such as *Mycobacterium tuberculosis*. Monocytes are further able to bind and destroy cells coated with complement-fixing antibodies because of the presence of membrane receptors for specific components or types of immunoglobulin.

Release of lytic enzymes results in the destruction of neutrophils and their subsequent phagocytosis by macrophages. Macrophage digestion proceeds without risk to the cell unless the ingested material is toxic. If the ingested material damages the lysosomal membrane, however, the macrophage will also be destroyed because of the release of lysosomal enzymes.

During phagocytosis, cells demonstrate increased metabolic activity, referred to as a respiratory burst. This results in the production by the phagocyte of large quantities of **reactive oxygen species (ROS),** which are released into the phagocytic vesicle. This phenomenon is achieved by the activity of the enzyme known as reduced nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase. Together, the granule-mediated and NADPH oxidase–mediated effects elicit microbicidal results. NADPH oxidase forms the centerpiece of the phagocyte-killing mechanism and is activated in about 2 seconds. The NADPH oxidase generates ROS by generating the superoxide radical (O_2^-); the associated cyanide-insensitive increase in oxygen consumption is the respiratory burst.

The importance of the oxygen-dependent microbicidal mechanism is dramatically illustrated by patients with chronic granulomatous disease (CGD), a severe congenital deficit in bacterial killing that results from the inability to generate phagocyte-derived superoxide and related reactive oxygen intermediates (ROIs). The production of residual ROIs is predicted by the specific NADPH oxidase mutation, regardless of the specific gene affected. CGD results from defects in the genes encoding individual components of the enzyme system responsible for oxidant production. Acquisition of oxidase activity occurs in the course of myeloid cell maturation, and the genes for several of its components have been identified. This system also lends itself to analysis of the transcriptional and translational events that occur during cellular differentiation and under the influence of specific cytokines.

Rather than being discarded by **exocytosis,** some peptides undergo an important separate process at this stage. Instead of being eliminated, they attach to a host molecule called major histocompatibility complex (MHC) class II and are expressed on the surface of the cell within a groove on the MHC molecule (antigen presentation).

Subsequent Phagocytic Activity

If invading bacteria are not phagocytized at entry into the body, they may establish themselves in secondary sites such as the lymph nodes or various body organs. These undigested bacteria produce a secondary inflammation, where neutrophils and macrophages again congregate. If bacteria escape from secondary tissue sites, a **bacteremia** will develop. In patients who are unresponsive to antibiotic intervention, this situation can prove fatal.

Neutrophil Extracellular Traps

In addition to phagocytosis, including the release of antimicrobial molecules at the site of infection, an additional defense mechanism has been discovered. This mechanism is the formation of **neutrophil extracellular traps (NETs),** which are produced following the release of the nuclear contents of the neutrophil into the extracellular space. NETs function in innate immunity. They are composed of chromatin components, including histones, and neutrophil antimicrobial proteins. Microbes are trapped in NETs, where they encounter high concentrations of antimicrobial proteins.

MONOCYTES-MACROPHAGES

In the past, the mononuclear monocyte-macrophage was known only as a scavenger cell. Only recently has its role as a complex cell of the immune system in the host defense against infection been recognized.

Mononuclear Phagocyte System

The macrophage (Fig. 3-4) and its precursors are widely distributed throughout the body. These cells constitute a physiologic system, the mononuclear phagocyte system (previously referred to as the reticuloendothelial system), which includes promonocytes and their precursors in the bone marrow, monocytes in the circulating blood, and macrophages in tissues. This collection of cells is considered to be a system because of the common origin, similar morphology, and shared functions, including rapid phagocytosis mediated by receptors for IgG and the major fragment of C3.

Macrophages and monocytes (see Color Plate 7) migrate freely into the tissues from the blood to replenish and reinforce the macrophage population. Cells of the macrophage system originate in the bone marrow from the multipotential stem cell. This common committed progenitor cell can differentiate into the granulocyte or monocyte-macrophage pathway, depending on the microenvironment and chemical regulators. Maturation and differentiation of these cells may occur in various directions. Circulating monocytes may continue to be multipotential and give rise to different types of macrophages.

Macrophages exist as fixed or wandering cells. Specialized macrophages such as the pulmonary alveolar macrophages are the so-called dust phagocytes of the lung that function as the first line of defense against inhaled foreign particles and bacteria. Fixed macrophages line the endothelium of capillaries and the sinuses of organs such as the bone marrow, spleen, and lymph nodes. Macrophages, along with the network of reticular cells of the spleen, thymus, and other lymphoid tissues, are organized into the mononuclear phagocyte system (Fig. 3-5).

Functionally, the most important step in the maturation of macrophages is the cytokine-driven conversion of the normal resting macrophage to the activated macrophage. Macrophages can be activated during infection by the release of macrophage-activating cytokines such as interferon-gamma (IFN-γ) and granulocyte colony-stimulating factor (G-CSF)

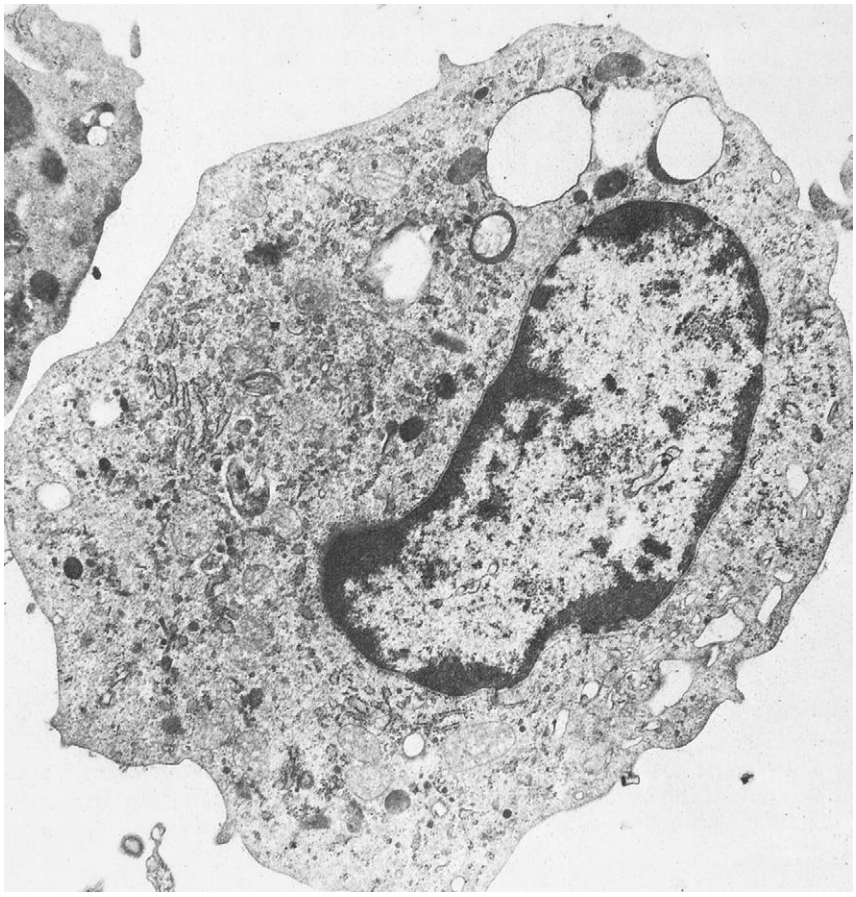


Figure 3-4 Electron micrograph of a macrophage. *(From Barrett JT: Textbook of immunology, ed 5, St Louis, 1988, Mosby.)*

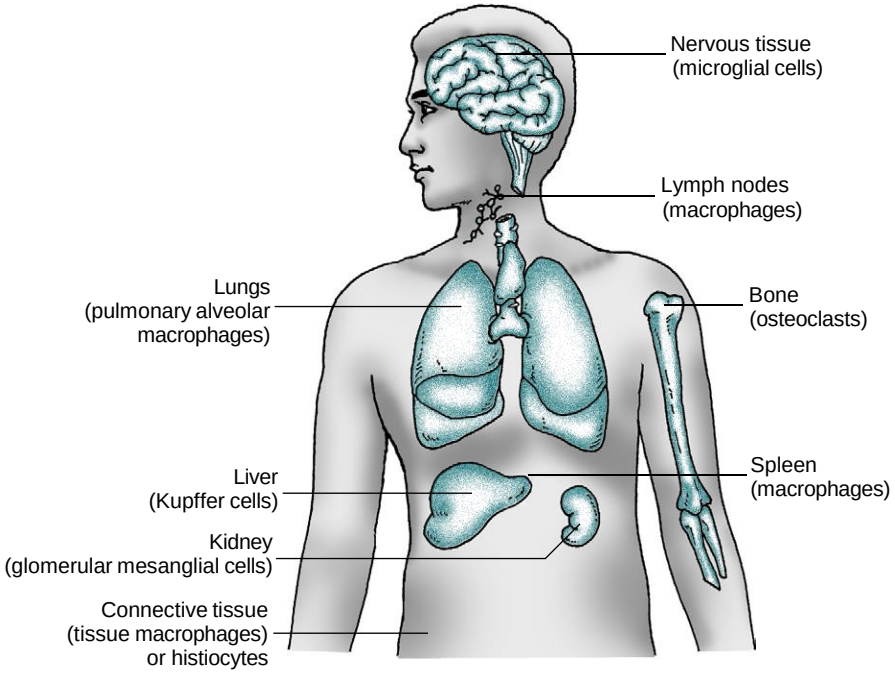


Figure 3-5 Mononuclear phagocyte system. *(Adapted from Roitt IM: Essential immunology, ed 5, Oxford, 1984, Blackwell Scientific.)*

from T lymphocytes specifically sensitized to antigens from the infecting microorganisms. This interaction constitutes the basis of cell-mediated immunity. In addition, macrophages exposed to an **endotoxin** release a hormone, tumor necrosis factor α (TNF-α, cachectin), which can activate macrophages itself under certain in vitro conditions.

The terminal stage of development in the mononuclear phagocyte cell line is the multinucleated giant cell, which characterizes granulomatous inflammatory diseases such as tuberculosis. Both monocytes and macrophages can be shown in the lesions in these diseases before the formation of giant cells, thought to be precursors of the multinucleated cells.

Host Defense Functions

Functionally, monocytes-macrophages have phagocytosis as their major role, but these cells perform at least three distinct but interrelated functions in host defense. The categories of host defense functions of monocytes-macrophages include phagocytosis, antigen presentation and induction of the immune response, and secretion of biologically active molecules.

Phagocytosis

The principal functions of mononuclear phagocytes in body defenses result from the changes that take place in these functions when the macrophage is activated (Box 3-1). Macrophages carry out the fundamental function of ingesting and killing invading microorganisms such as intracellular parasites, *M. tuberculosis,* and some fungi. In addition, macrophages remove and eliminate such extracellular pathogens as pneumococci from the blood circulation. The macrophage also has the capacity to phagocytize particulate and aggregated soluble materials. This process is enhanced by the presence of receptors on the surface of the Fc portion of IgG and C3. The ability to internalize soluble substances supports the increased microbicidal and tumoricidal ability of activated macrophages. Activation of macrophages or monocytes can result in the release of parasiticidal mediators and in receptor-mediate phagocytosis during malaria infection. The most likely location for this innate immune response is within the spleen which is crucial for development of immunity to malaria.

Another important phagocytic function of macrophages is their ability to dispose of damaged or dying cells. Macrophages lining the sinusoids of the spleen are particularly important in ingesting aging erythrocytes. They are also involved in removing tissue debris, repairing wounds, and removing debris as embryonic tissues replace one another.

Phagocytic activity increases when there is tissue damage and inflammation, which releases substances that attract macrophages. Activated macrophages migrate more vigorously in response to chemotactic factors and should enter sites of inflammation (e.g., locations of infection or cancer) more efficiently than resting macrophages. Migration of monocytes into different body tissues appears to be a random phenomenon in the absence of localized inflammation. An essential factor in the protective function of monocytes is the capacity of the cell to move through the endothelial wall of blood vessels (diapedesis) to the site of microbial invasion in tissues. The attracting forces for monocytes, chemotactic factors, include complement products and chemoattractants derived from neutrophils, lymphocytes, or cancer cells.

Box 3-1 Functions of Mononuclear Phagocytes

Increased Activity in Activated Macrophages
Antigen presentation
Chemotaxis
Glucose transport and metabolism
Microbicidal activity
Phagocytosis (variable activity, depending on particle)
Phagocytosis-associated respiratory burst
Pinocytosis
Tumoricidal activity

Increased Constituents in Activated Macrophages
Acid hydrolases
Angiogenesis factor
Arginase
Collagenase
Complement components*
Cytolytic proteinase
Fibronectin
Interleukin-1
Interferon (α and β)
Plasminogen activator
TNF-α (cachectin)†

Decreased Constituents in Activated Macrophages
Apolipoprotein E and lipoprotein lipase
Elastase
Prostaglandins, leukotrienes

Constituent Demonstrating No Change in Activated Macrophages
Lysoenzyme

Adapted from Johnston RB: Current concepts: immunology. Monocytes and macrophages, N Engl J Med 318:747–752, 1988.
*Increased or no change.
†When stimulated.

The activity of mononuclear phagocytes against cancer cells in humans is less well understood than the phagocytosis of microorganisms. Phagocytes are thought to suppress the growth of spontaneously arising tumors. The ability of these cells to control malignant cells may not involve phagocytosis but may be related to secreted cellular products such as lysosomal enzymes, oxygen metabolites (e.g., H_2O_2), proteinases, and TNF-α (cachectin). The proteolytic enzymes present on the surface membrane of monocytes also may play a role in tumor rejection.

Antigen Presentation and Induction of the Immune Response

The phagocytic property of the macrophage is particularly important in the processing of antigens as part of the immune response. Macrophages are believed to process antigens and physically present this biochemically modified and more reactive form of antigen to lymphocytes (particularly helper T cells) as an initial step in the immune response. Recognition of

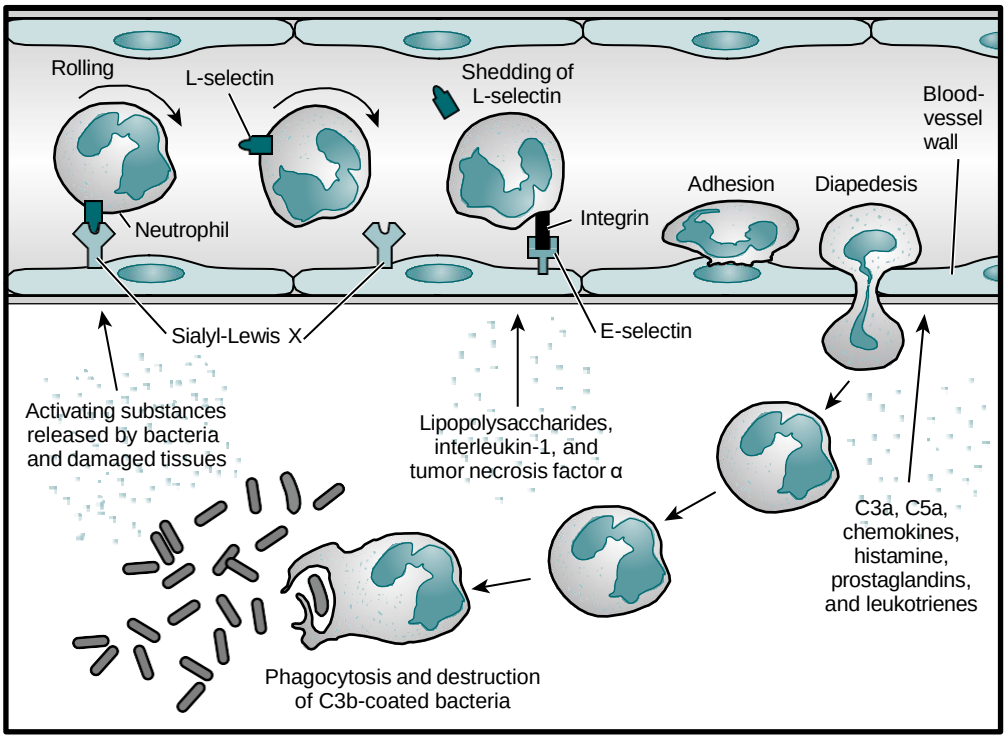


Figure 3-6 Acute inflammatory response. Neutrophils are among the first cells to arrive at the scene of an infection and are important contributors to the acute inflammatory response. As the neutrophil rolls along the blood vessel wall, the L-selectin on its surface binds to carbohydrate structures such as sialyl-Lewis X on the adhesion molecules on the vascular endothelium, and its progress is eventually halted. As the neutrophil becomes activated, it replaces L-selectin with other cell surface adhesion molecules, such as integrins. These molecules bind E-selectin, which is present on the blood vessel wall as a result of the influence of inflammatory mediators such as bacterial lipopolysaccharides and the cytokines IL-1 and TNF-α. The activated neutrophil then enters the tissues, where it is attracted to the infection site by a number of chemoattractants. The neutrophil can then phagocytose and destroy the C3b-coated bacteria. *(Adapted from Delves PJ, Roitt IM: The immune system. First of two parts, N Engl J Med 343:37–49, 2000.)*

antigen on the macrophage surface by T lymphocytes, however, requires an additional match of the surface MHC class II gene product. This gene product is the Ia product in the mouse and D gene region product in humans. With proper recognition, the macrophage secretes a lymphocyte-activating factor (IL-1), lymphocyte proliferation ensues, and the immune response (T cell–B cell response) is facilitated.

Secretion of Biologically Active Molecules

Monocytes-macrophages release many factors associated with host defense and inflammation. These cells serve as supportive accessory cells to lymphocytes, at least partly by releasing soluble factors. In cellular immunity, monocytes assume a killer role in that they are activated by sensitized lymphocytes to phagocytize offending cells or antigen particles. This is important in fields such as tumor immunology.

In addition to their phagocytic properties, monocytes-macrophages are able to synthesize a number of biologically important compounds, including transferrin, complement, **interferon,** pyrogens, and certain growth factors. Approximately 100 distinct substances have been identified as being secreted by monocytes-macrophages.

Blood monocytes and tissue macrophages are primary sources of the polypeptide hormone called IL-1, which has a particularly potent effect on the inflammatory response. IL-1 also supports B lymphocyte proliferation and antibody production, as well as T lymphocyte production of lymphokines. The increased synthesis of IL-1 by activated macrophages could contribute to enhancement of the immune response. Endotoxin also induces the synthesis of IL-1. This effect is achieved at least partly by stimulation of the macrophages to release TNF-α, which then stimulates the production of IL-1 by endothelial cells and macrophages. Activated macrophages release much more TNF-α than resting macrophages exposed to endotoxin. Both TNF-α and IL-1 can induce the fever and synthesis of acute-phase reactants that characterize inflammation.

ACUTE INFLAMMATION

Tissue damage results in inflammation, a series of biochemical and cellular changes that facilitate the phagocytosis of invading microorganisms or damaged cells (Fig. 3-6). If inflammation is sufficiently extensive, it is accompanied by an increase in the plasma concentration of acute-phase reactants (see Chapter 5). Leukocyte recruitment into inflamed tissue follows a well-defined cascade of events beginning with the capture of free-flowing WBCs to the vessel wall and subsequent leukocyte rolling along and adhesion to the inflamed endothelial layer. During rolling, WBCs come into close contact with the endothelial surface, which allows endothelium-bound chemokines

to interact with their specific receptors on the leukocyte surface. This triggers the activation of integrins, which leads to firm leukocyte arrest on the endothelium. In addition, integrin-dependent signaling events induce cytoskeletal rearrangements and cell polarization, modifications necessary to help prepare the attached leukocyte to spread and crawl in search for a way out of the vasculature into tissue.

Celsus, a practitioner of Greek medicine who was born in 25 BCE, is credited with recording the cardinal signs of inflammation—rubor (redness), calor (heat), dolor (pain), and tumor (swelling). The primary objective of inflammation is to localize and eradicate the irritant and repair the surrounding tissue. The inflammatory response involves the following three major stages:

1. Dilation of capillaries to increase blood flow
2. Microvascular structural changes and escape of plasma proteins from the bloodstream
3. Leukocyte transmigration through endothelium and accumulation at the site of injury

Hypoxia can induce inflammation. Inflammation in response to hypoxia is clinically relevant. Ischemia in organ grafts increases the risk of inflammation and graft failure or rejection. Hypoxia has multiple effects on the innate and adaptive immune systems.

Once inflammation is triggered, it must be appropriately resolved or pathologic tissue damage will occur. In some diseases, the body's defense system (immune system) inappropriately triggers an inflammatory response when no foreign substances are present. In these autoimmune disorders, the body's normally protective immune system causes damage to its own tissues (see Chapter 28).

SEPSIS

If an inflammation overwhelms the whole body, systemic inflammatory response syndrome (SIRS) is diagnosed. Sepsis, severe sepsis, and septic shock are progressively severe stages of SIRS. The criteria for SIRS require two or more conditions: alteration of body temperature (>38°C or <36°C), increased heart rate, increased respiratory rate, and a total leukocyte count of >12.0 × 10(9)/L (or >10% immature forms). Sepsis is defined as SIRS + infection; severe sepsis is defined as sepsis + evidence of organ dysfunction. Patients with severe sepsis are considered to have defective adaptive immunity.

Sepsis begins when the innate immune system responds aggressively to the presence of bacteria. Toll-like receptors (TLR) cause the antigen presenting cell (APC) to produce proinflammatory cytokines. Biochemical markers associated with sepsis include tumor necrosis factor (TNF) and interleukins (ILs) IL-1 and IL-6, a proinflammatory cytokine. Other proteins produced in response infection and/or inflammation include procalcitonin and chemokine production. Another consequence of inflammation is that the liver is stimulated to produce C-reactive protein (C-RP) (see Chapter 5).

These cytokines produce systemic inflammation by activating circulating polymorphonuclear leukocytes (PMNs). APCs involve the adaptive immune system by presenting bacterial antigen to T-cell receptor using a Class II major histocompatibility complex (MHC) protein and co-stimulation of CD28.

CELL SURFACE RECEPTORS

Cellular communication is essential to the development, tissue organization, and function of all multicellular organisms. Cells communicate with each other and their environment through soluble mediators and during direct contact (e.g., phagocytosis). An immunologic response is a result of the interactions of various leukocytes with each other and other cells in the body. These interactions occur through **cell surface receptors** that mediate cell-cell binding, or adhesion, of leukocytes.

The discovery of several cell surface receptors involved in cellular communication has been a key factor in understanding the mechanisms underlying inflammatory and immune phenomena. Three protein families—the immunoglobulin (Ig) family, integrin family, and the rather recently designated **selectin** family—form a network of cellular interactions in the immune system. Neutrophil tether to and roll on P- and E-selectin expressed on activated endothelial cells. Rolling neutrophils encounter immobilized chemokines. **Chemokines** activate integrins to their high-affinity states that enable interactions with intercellular adhesion molecule-1 (ICAM-1), which promote arrest, adhesion strengthening, intraluminal crawling, and transendothelial migration. E-selectin directly triggers signals in rolling PMN that cooperate with chemokine signals to minimize neutrophil recruitment during inflammation.

Members of the Ig superfamily include antigen-specific receptors (e.g., T cell receptor [TCR] and surface immunoglobulin [sIg]), as well as antigen-independent receptors and their counterreceptors, such as CD2 and lymphocyte function–associated antigen-3. Ig superfamily members function in cell activation, differentiation, and cell-cell interaction. In some cases, both an adhesion receptor and the counterreceptor to which it binds are members of the Ig superfamily.

Three selectin family molecules—endothelial cell adhesion molecule-1, leukocyte adhesion molecule (LAM-1, Mel-14), and CD62, also known as platelet activation–dependent granule–external membrane protein and granule membrane protein of 140 kDa (GMP-140)—have been implicated in a number of leukocyte adhesion phenomena, including leukocyte homing to lymphoid tissue. Selectins are expressed on leukocytes and endothelial cells. Mel-14 functions early in neutrophil-endothelium adhesion.

The integrin family consists of at least 14 alpha-beta heterodimers divided into subfamilies with distinct structural and functional characteristics. The subfamily of **leukocyte integrins** contains three members—LFA-1, Mac-1, and p150,95. These molecules are glycoproteins composed of noncovalently associated alpha and beta subunits. LFA-1 is expressed on all leukocytes, whereas Mac-1 and p150,95 are found primarily on granulocytes and monocytes.

The integrin family is phylogenetically ancient. Integrin family members engage in interactions with cell surface ligands and **extracellular matrix (ECM)** components. ECM components, including fibronectin, collagen, and laminin, have been shown to be ligands for members of the beta-1 and beta-3 subfamilies. Members of these subfamilies are of great

significance in embryogenesis, growth and repair, and hemostasis. The leukocyte integrins, or beta-2 subfamily, have been shown to be involved in a diverse number of leukocyte adhesion–dependent phenomena, giving them a critical role in inflammatory and immune responses. The term *integrin* was initially used to emphasize that these receptors integrate signals from the extracellular environment with the intracellular cytoskeleton. A signal is transduced from outside to inside the cell.

In addition to the involvement of these receptors in a variety of immune functions, integrin molecules play a role in the spread of malignant cells. The major cause of death in malignant disease is not the primary tumor but rather the metastasis of tumor cells to distant sites within the body. Metastasis is a complex multistep process that begins with the detachment of a few tumor cells from the primary tumor. The tumor cells then move into the circulatory system, where they can be transported to other organs. While in the circulatory system, tumor cells must survive the natural defense system of the body before attaching to and invading the tissues of another organ. A better understanding of the metastatic process could provide the basis for diagnostic and therapeutic strategies.

DISORDERS OF NEUTROPHILS

Noninfectious Neutrophil-Mediated Inflammatory Disease

Although neutrophils provide the major means of defense against bacterial and fungal infections, they can also be destructive to host tissues. The same oxidative and nonoxidative processes that destroy microorganisms can affect adjacent host tissues. A number of disease states correspond to inappropriate phagocytosis (Box 3-2), as with prolonged activation of NADPH oxidase. This process occurs when phagocytes attempt to engulf particles that are too large. The phagocyte releases oxygen radicals and granule contents onto the particle, but these escape into the surrounding tissues, generating tissue damage. This is often observed in response to dust inhalation and smoking (e.g., nicotine) and in persistent infections such as cystic fibrosis. In addition, many autoimmune diseases are thought to be caused by inappropriate activation of the process of phagocytosis, whereby the body attacks its own cells and tissues. Examples include rheumatoid arthritis, multiple sclerosis, and Graves' disease.

Abnormal Neutrophil Function

Patients with quantitative or qualitative defects of neutrophils have a high rate of infection, which illustrates the importance of the neutrophil to body defenses. Individuals with a marked decrease of neutrophils (neutropenia) or severe defects in neutrophil function frequently have recurrent systemic bacterial infections (e.g., pneumonia), disseminated cutaneous pyogenic lesions, and other types of life-threatening bacterial and fungal infections.

Leukocyte mobility may be impaired in some diseases (e.g., rheumatoid arthritis, cirrhosis, CGD). Defective locomotion or leukocyte immobility can also be seen in patients receiving steroids and in those with lazy leukocyte syndrome. A marked defect in the cellular response to chemotaxis, an important step in phagocytosis, can be seen in patients with diabetes mellitus, Chédiak-Higashi anomaly (syndrome), or sepsis, as well as in those with high levels of antibody immunoglobulin E (IgE), as in Job's syndrome.

Congenital Neutrophil Abnormalities

A small number of patients have congenital abnormalities of neutrophil structure and function (Box 3-3).

Chédiak-Higashi Syndrome

The **Chédiak-Higashi syndrome** represents a qualitative disorder of neutrophils. It is a rare familial disorder inherited as an autosomal recessive trait and expressed as an abnormal granulation of neutrophils. Neutrophils having giant granules display impaired chemotaxis and delayed killing of ingested bacteria.

Chronic Granulomatous Disease

The **chronic granulomatous diseases (CGDs)** are a genetically heterogeneous group of disorders of oxidative metabolism affecting the cascade of events required for H_2O_2 production by phagocytes. Patients with X-linked CGD (X-CGD) have a mutation in CYBB encoding the transmembrane $gp91^{phox}$ subunit of phagocyte NADPH oxidase

Box 3-2 Noninfectious Neutrophil-Mediated Diseases*

- Autoimmune arthritides
- Autoimmune vasculitis
- Dermatophytic disorders
  - Autoimmune bullous dermatoses
  - Behçet's disease
  - Psoriasiform dermatoses
  - Pyoderma gangrenosum
  - Sweet's syndrome
- Glomerulonephritis
- Gout
- Inflammatory bowel disease
- Malignant neoplasms at site of chronic inflammation
- Myocardial infarction
- Respiratory disorders
  - Adult respiratory distress syndrome
  - Asthma and allergic asthma
  - Emphysema

*Signs, symptoms, and injury may be partly mediated by neutrophils.

Box 3-3 Congenital Neutrophil Abnormalities

- Chédiak-Higashi syndrome (anomaly)
- Chronic granulomatous disease (CGD)
- Complement receptor 3 (CR3) deficiency
- Myeloperoxidase deficiency
- Specific granule deficiency

required for microbicidal ROS production by neutrophils and monocytes. As a result, patients have life-threatening infections and granulomatous complications. If a suitable hematopoietic stem cell donor is available, it can cure X-CGD, but graft-versus-host disease (see Chapter 31) is a significant risk.

A number of types of inheritance of the disorder have been described, including sex-linked (X chromosome–linked) in 66%, autosomal recessive in 34%, and autosomal dominant in less than 1% of cases. Patients with the autosomal recessive form may have a less severe clinical course than patients with the X-linked form. CGD is a defect of neutrophil microbicidal ROS generation resulting from gp91phox deficiency. CGD is caused by a missense, nonsense, frameshift, splice, or deletion mutation in the genes for p22 phox, p40 phox, p47 phox, p67 phox (autosomal CGD), or gy91phox (X-linked CGD), which results in variable production of neutrophil-derived ROIs.

The onset of CGD is during infancy, with one third of patients dying before the age of 7 years because of infections. It was observed that in the presence of normal or elevated leukocyte counts, the neutrophilic granulocytes in vitro ingested and destroyed only streptococci, not staphylococci. Subsequent testing revealed that cells from patients with CGD can phagocytize non–H_2O_2-producing bacteria such as *Staphylococcus aureus* and gram-negative rods (e.g., Enterobacteriaceae), but cannot destroy them. In the X-linked form, the defective leukocytes fail to exhibit increased anaerobic metabolism during phagocytosis because of a cytochrome b558 deficiency (which expresses itself as a defect in the 91,000-Da glycoprotein membrane anchor of the cytochrome complex), or these defective leukocytes produce H_2O_2 because of a myeloperoxidase deficiency.

Patients with CGD have infections with catalase-positive bacteria and fungi affecting the skin, lungs, liver, and bones. They also develop granulomas, resulting from a lack of resolution of inflammatory foci, even after the infection has been eliminated. This leads to extensive granuloma formation and, in some circumstances, impairment of physiologic processes (e.g., obstruction of the esophagus or urinary tract).

Laboratory evaluation of CGD begins with non-specific testing to rule out other disorders. These assays include serum quantitative immunoglobulin, complement activity enzyme immunoassay, CBC with differential, myeloperoxidase stain and a neutrophil receptor profile. The evaluation of neutrophil phagocytic function is best determined by the neutrophil oxidative burst assay (DHR) via flow cytometry (see Appendix C) that can indicate CGD by the absence or significant alteration of activity. Other, less-reliable tests include measurement of superoxide production, ferrocytochrome reduction, and the classic nitroblue tetrazolium test (NBT).

Complement Receptor 3 Deficiency

The complement receptor 3 (CR3) deficiency is a rare condition inherited as an autosomal recessive trait. A deficiency of CR3 on phagocytic cells presents as a leukocyte adhesion deficiency. Leukocyte adhesion deficiency type 1 (LAD-1) is caused by a deficiency of CD18. LAD-2 is caused by the absence of sialyl–Lewis X (CD15s) blood group antigen.

A CR3 deficiency in neutrophils is associated with marked abnormalities of adherence-related functions, including decreased aggregation of neutrophils to each other after activation, decreased adherence of neutrophils to endothelial cells, poor adherence and phagocytosis of opsonized microorganisms, defective spreading, and decreased diapedesis and chemotaxis. Patients may also lack an intravascular marginating pool of neutrophils. Defects in T lymphocytes are characterized by faulty lymphocyte-mediated cytotoxicity, with poor adherence to target cells. Abnormalities of B lymphocytes have also been observed.

Clinically, a deficiency can manifest as delayed separation of the umbilical cord. Other signs and symptoms include early onset of bacterial infections, including skin infections, mucositis, otitis, gingivitis, and periodontitis. A depressed inflammatory response and neutrophilia can be observed.

Myeloperoxidase Deficiency

A deficiency of myeloperoxidase is inherited as an autosomal recessive trait on chromosome 17. Myeloperoxidase is an iron-containing heme protein responsible for the peroxidase activity characteristic of azurophilic granules; it accounts for the greenish color of pus. Human neutrophils contain many granules of various sizes that are morphologically, biochemically, and functionally distinct. The azurophilic granules normally contain myeloperoxidase. In this disorder, azurophilic granules are present, but myeloperoxidase is decreased or absent. If phagocytes are deficient in myeloperoxidase, the patient's phagocytes manifest a mild to moderate defect in bacterial killing and a marked defect in fungal killing in vitro.

Persons with a myeloperoxidase deficiency are generally healthy and do not have an increased frequency of infection, probably because of other microbicidal mechanisms compensating for the deficiency. Patients with diabetes and myeloperoxidase deficiency, however, may have deep fungal infections caused by *Candida* spp.

Specific Granule Deficiency

Specific granule deficiency is believed to be an autosomal recessive disease. It is caused by a failure to synthesize specific granules and some contents of other granules during differentiation of neutrophils in the bone marrow. Patients with specific granule deficiency have recurrent, severe bacterial infections of the skin and deep tissues, with a depressed inflammatory response.

MONOCYTE-MACROPHAGE DISORDERS

Monocytes-macrophages have been shown to be abnormal in a variety of diseases (Table 3-2). The abnormality is partial and no related association with increased susceptibility to infection has been established. In cases of severely depressed migration of monocytes, however, it is likely that this dysfunction

Table 3-2 Primary and Secondary Abnormalities of Monocyte-Macrophage Function

Abnormality	Condition/Group
Defect in phagocyte killing	Chronic granulomatous disease, corticosteroid therapy, newborn infants, viral infections
Defective monocyte cytotoxicity	Cancer, Wiskott-Aldrich syndrome
Defective release of macrophage-activating factors	Acquired immunodeficiency syndrome (AIDS), intracellular infections (e.g., lepromatous leprosy, tuberculosis, visceral leishmaniasis)
Depressed migration	AIDS, burns, diabetes, immunosuppressive therapy, newborn infants
Impaired phagocytosis	Congenital deficiency of CD11 to CD18, monocytic leukemia, systemic lupus erythematosus

predisposes a patient to infection because other defects of host defense coexist in these disorders.

The signs and symptoms of abnormalities of monocyte-macrophage function are extremely evident in some conditions. The profound defect of phagocytic killing exhibited by patients with CGD results in the formation of subcutaneous abscesses and abscesses in the liver, lungs, spleen, and lymph nodes. Cancer patients with a defective monocyte cytotoxicity may develop this defect because tumors have the ability to release factors that suppress the generation of toxic oxygen metabolites by macrophages. In newborn infants, depressed chemotaxis, killing, and decreased synthesis of the phagocytosis-promoting factors fibronectin, C3, and complement factor B have been observed. In addition, the newborn's macrophages may not respond effectively to infection because the lymphocytes have impaired the production of the macrophage activator IFN-γ.

Qualitative disorders of monocytes-macrophages manifest as lipid storage diseases, including a number of rare autosomal recessive disorders. The expression in macrophages of a systemic enzymatic defect permits the accumulation of cell debris normally cleared by macrophages. The macrophages are particularly prone to accumulate undegraded lipid products. Resistance to infection can be impaired, at least partially, because of a defect in macrophage function. Disorders of this type include Gaucher's disease and Niemann-Pick disease.

Gaucher's Disease

An inherited disease caused by a disturbance in cellular lipid metabolism, **Gaucher's disease** most frequently affects children. The prognosis varies; with mild disease, the patient may live a relatively normal life, whereas with severe disease the patient may die prematurely.

Thedisorderrepresents a deficiencyof β-glucocerebrosidase, the enzyme that normally splits glucose from its parent sphingolipid, glucosylceramide. As a result of this enzyme deficiency, cerebroside accumulates in histiocytes (macrophages). Gaucher's cells are rarely found in the circulating blood; the typical cell is large, with one to three eccentric nuclei and a characteristically wrinkled cytoplasm. These cells are found in the bone marrow, spleen, and other organs of the mononuclear phagocyte system. Production of erythrocytes and leukocytes decreases as these abnormal cells infiltrate the bone marrow.

Niemann-Pick Disease

Niemann-Pick disease is similar to Gaucher's disease, also an inherited abnormality of lipid metabolism. Niemann-Pick disease affects infants and children, with an average life expectancy of 5 years.

This disorder represents a rare autosomal recessive deficiency of the enzyme sphingomyelinase, characterized by massive accumulation of sphingomyelin in the mononuclear phagocytes. The characteristic cell in this disorder, Pick's cell, is similar in appearance to Gaucher's cell, although the cytoplasm of the cell is foamy.

DISEASE STATES INVOLVING LEUKOCYTE INTEGRINS

Leukocyte adhesion deficiency (LAD) ultimately leads to recurrent and often fatal bacterial and fungal infections. The cause of this very rare condition is mutations in the gene or chromosome; about 300 cases have been diagnosed worldwide.

There are several types of LAD based on genotypes and phenotypes. Two genotypes have been identified, LAD-1 and LAD-2. LAD-1 can affect people of all racial groups. LAD-2 has been reported only in people from the Middle East and Brazil. LAD-1 patients have a deficiency of the β_2-integrin subunit (CD18). The phenotypes are severe, moderate, and novel or variant. LAD-2 is described as the failure to convert guanosine diphosphate (GDP) mannose to fructose.

Patients have a history of delayed separation of the umbilical cord, gingivitis, recurrent and persistent bacterial or fungal skin infections, and impaired wound healing. A lack of pus formation has also been noted. Patients frequently develop severe life-threatening infections, although their neutrophil counts are usually elevated (25.0×10^9/L). Affected individuals do not have increased susceptibility to viral infections or malignant neoplasms. Patients with LAD-2 have a characteristic facial appearance, short stature, limb malformations, and severe developmental delay.

Adhesion defects can also be caused by two common drugs, epinephrine and corticosteroids. Both demarginate neutrophils from the peripheral vasculature, although the mechanism is not understood. Epinephrine acts by causing endothelial cells to release cyclic adenosine monophosphate (cAMP), which in turn interrupts adherence.

CASE STUDY 1

History and Physical Examination

This family had a son who had died at age 2 weeks because of overwhelming bacterial infection. When their newborn daughter began developing recurrent infections, she was immediately taken to a pediatrician.

Laboratory Data

Hemoglobin and hematocrit—within normal range
Total WBC count—62.0 × 10^9/L
Absolute leukocyte counts—above normal for each leukocyte type
Leukocyte differential—neutrophils 76%, lymphocytes 22%, eosinophils 2%
Flow cell cytometry
- T lymphocytes—normal proportions of CD4+ and CD8+ cells
- B lymphocytes (XΔ19+)—elevated
- Natural killer (NK) cells—elevated
- CD15+ lymphocytes—absent

Serum Ig fractions—within reference ranges

Treatment

The infant was given busulfan cyclophosphamide and antithymocyte serum for 10 days. She received mature T lymphocyte–depleted bone marrow transplanted from her mother. This was followed by a short period of immunosuppressive therapy.

She recovered from the procedures and did well clinically.

Questions

1. What significant finding in flow cytometry suggests an immune deficiency?
   a. Elevated B-lymphocytes (CD19+) count.
   b. Normal CD4+ and CD8+ lymphocyte counts
   c. Elevated NK cell count
   d. Absence of CD15+ cells
2. What does the patient's family history suggest?
   a. Acute leukemia
   b. Immune antibody dysfunction
   c. Genetic leukocyte disorder
   d. Hereditary anemia

See Appendix A for the answers to these questions.

Critical Thinking Group Discussion Questions

1. What laboratory test is of the greatest diagnostic value in diagnosing this patient?
2. What value in the reported flow cytometry results is diagnostic?
3. Can leukocyte adhesion deficiency be misdiagnosed?

See instructor site on evolve for discussion of the answers to these questions.

CASE STUDY 2

History and Physical Examination

This 6-year-old white male patient was taken to a pediatrician because of recurring abscesses since the age of 1 month. The current abscesses were lanced and he was placed on antibiotic therapy.

The patient had two brothers who had died in infancy of infections. His parents and two sisters are healthy.

Laboratory Data

Hemoglobin and hematocrit—slightly decreased
Total leukocyte count—elevated
Differential leukocyte count—increased percentage of segmented neutrophils
Immunoglobulin profile—polyclonal elevation of all Ig classes
Neutrophil oxidative burst assay (DHR) activity absent
Nitroblue tetrazolium (NBT) test (automated)—reduction of unstimulated and stimulated neutrophils
Culture of abscess revealed *S. aureus*

Questions

1. What does the patient's family history suggest?
   a. A genetic disorder in male offspring
   b. A genetic disorder in female offspring
   c. Lack of leukocyte production
   d. Anemia producing an immune dysfunction

What laboratory assay is the most helpful in the diagnosis of this case?
   a. Percentage of segmented neutrophils
   b. Immunoglobulin profile
   c. Neutrophil oxidative burst assay (DHR)
   d. Nitroblue tetrazolium (NBT) test (automated)

See Appendix A for the answers to these questions.

Critical Thinking Group Discussion Questions

1. Does this boy's condition appear to be gender-related?
2. Why are the bacteria not killed?

See instructor site evolve for discussion of the answers to these questions.

Screening Test for Phagocytic Engulfment

Principle

A mixture of bacteria and phagocytes is incubated and examined for the presence of engulfed bacteria. This simple procedure may be useful in supporting the diagnosis of impaired neutrophilic function in conjunction with clinical signs and symptoms (Fig. 3-7).

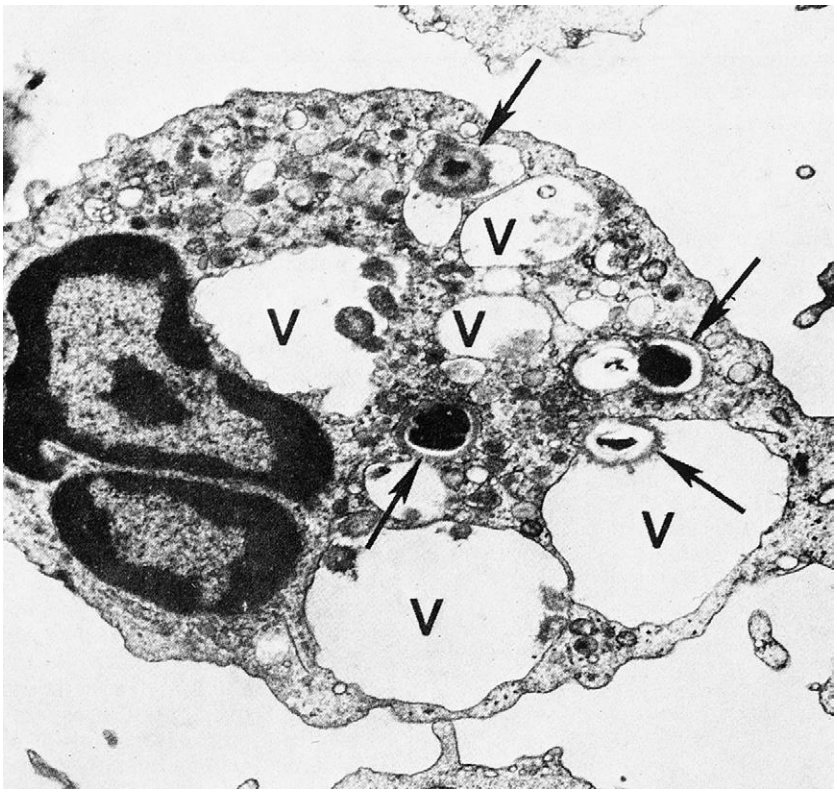


Figure 3-7 Electron photomicrograph of polymorphonuclear leukocyte from normal control patient incubated with staphylococci for 30 minutes. Many bacteria *(arrows)* in various stages of destruction are evident within the cell. Note the cytoplasmic vacuoles (V) around and adjacent to degenerating bacteria. *(From Bauer JD: Clinical laboratory methods, ed 9, St Louis, 1982, Mosby.)*

See Evolve website for information related to performing the procedure.

Reporting Results

- Positive—demonstration of the engulfment of bacteria
- Negative—no engulfment of bacteria

Sources of Error

This procedure may produce false-negative results if the blood specimen is not fresh or if a coagulase-positive *S. aureus* specimen is used. It is important to distinguish between granules and cocci. In addition, the bacteria must be intracellular and not extracellular for the test result to be positive.

Clinical Applications

The failure of phagocytes to engulf bacteria can support the diagnosis of neutrophilic dysfunction; however, these results must be used in conjunction with patient signs and symptoms.

Limitations

This is a simple screening procedure for engulfment. The presence of engulfed bacteria does not demonstrate that the bacteria have been destroyed.

CHAPTER HIGHLIGHTS

- The entire leukocytic cell system is designed to defend the body against disease. Each cell type has a unique function and behaves independently and, in many cases, in cooperation with other cell types.
- The primary phagocytic cells are the neutrophilic leukocytes and the mononuclear monocytes-macrophages.
- The neutrophilic leukocyte provides an effective host defense against bacterial and fungal infections. Although the monocytes-macrophages and other granulocytes are also phagocytic cells, the neutrophil is the principal leukocyte associated with phagocytosis and a localized inflammatory response.
- Phagocytosis can be divided into movement of cells, engulfment, and digestion.
- Cells communicate with each other and their environment through soluble mediators and during direct contact (e.g., phagocytosis). These interactions occur through cell surface receptors that mediate cell-cell binding (adhesion) of leukocytes.
- Three protein families (immunoglobulin, integrin, selectin) are associated in a network of cellular interactions in the immune system.
- Qualitative monocyte-macrophage disorders manifest as lipid storage diseases, including a number of rare autosomal recessive disorders.
- Leukocyte adhesion deficiency ultimately leads to recurrent and often fatal bacterial and fungal infections.

REVIEW QUESTIONS

1. The site of hematopoiesis in the first month of gestation is the:
   a. Yolk sac
   b. Spleen
   c. Liver
   d. Bone marrow

2. The principal type of leukocyte in the process of phagocytosis is the:
   a. Eosinophil
   b. Basophil
   c. Monocyte
   d. Neutrophil

3. Chronic granulomatous disease represents a defect of:
   a. Oxidative metabolism
   b. Abnormal granulation of neutrophils
   c. Diapedesis
   d. Chemotaxis

4. A primary function of the eosinophil is:
   a. Phagocytosis
   b. Suppression of the inflammatory response
   c. Reacting in acute, systemic hypersensitivity reactions
   d. Antigen recognition

5. The cells of the mononuclear phagocyte system include:
   a. Monocytes and promonocytes
   b. Monocytes and macrophages
   c. Lymphocytes and monocytes
   d. Both a and b

6. The host defense function(s) of monocytes-macrophages include(s):
   a. Antigen presentation
   b. Phagocytosis
   c. Secretion of biologically active molecules
   d. All of the above

7. The surface MHC class II gene product is important in:
   a. Antigen recognition by T lymphocytes
   b. Antigen recognition by B lymphocytes
   c. Synthesis of antibody by plasma cells
   d. Phagocytosis

8-12. Match the appropriate monocyte-macrophage abnormality with its respective condition.

8. _____ Defect in phagocytic killing

9. _____ Defective monocyte cytotoxicity

10. _____ Defective release of macrophage-activating factors

11. _____ Depressed migration

12. _____ Impaired phagocytosis
   a. Wiskott-Aldrich syndrome
   b. Burns or diabetes
   c. Systemic lupus erythematosus
   d. Corticosteroid therapy
   e. Intracellular infections

13-16. Arrange the steps of phagocytosis in the proper sequence.

13. _____

14. _____

15. _____

16. _____
   a. Digestion of bacteria
   b. Increase in chemoattractants at site of tissue damage
   c. Ingestion of bacteria
   d. Movement of phagocytic cells

17-20. Match the following cell types to their respective functions. (An answer can be used more than once.)

17. _____ Polymorphonuclear neutrophil (PMN) leukocytes

18. _____ Lymphocytes

19. _____ Mononuclear monocytes-macrophages

20. _____ Plasma cells
   a. Primary phagocytic cells
   b. Antibody-synthesizing cells
   c. Recognition of foreign antigen and production of antibody

21-23. Arrange the sites of blood cell development (hematopoiesis) in the embryo and fetus in the correct sequence of development.

21. _____ Site of initial red blood cell production

22. _____ Predominant site from 2 to 5 months of fetal life

23. _____ Ultimate site of primary hematopoiesis
   a. Liver and spleen
   b. Yolk sac
   c. Bone marrow

24. Patients with a marked decrease in neutrophils or severe defects in neutrophil function have:
   a. A high rate of infection
   b. Recurrent systemic bacterial infections
   c. Recurrent life-threatening fungal infections
   d. All of the above

25-28. Match each disorder or deficiency to its characteristics.

25. _____ Chronic granulomatosus disease

26. _____ Lazy leukocyte syndrome

27. _____ Chédiak-Higashi anomaly (syndrome)

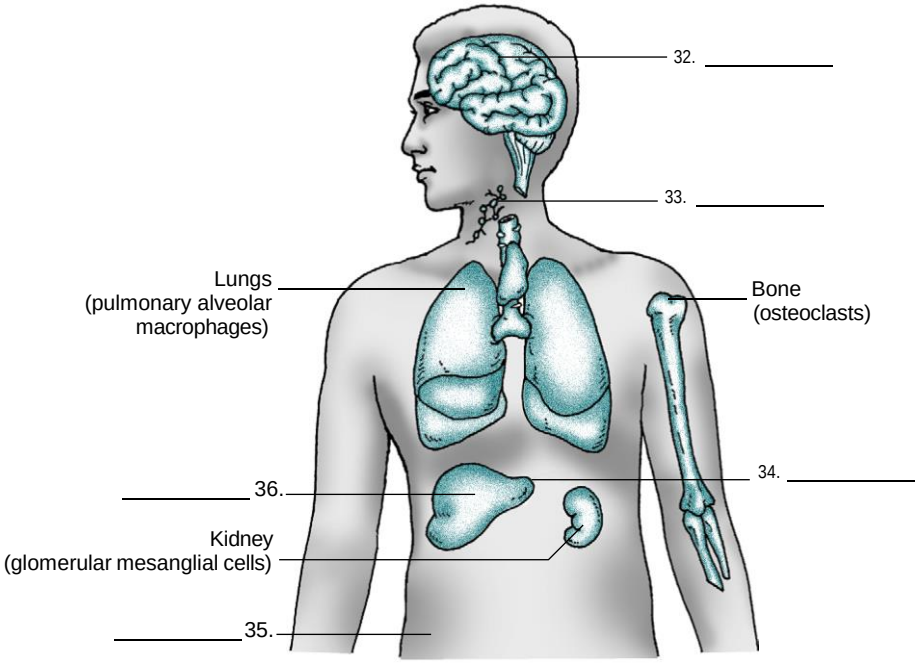
28. _____ Myeloperoxidase deficiency
   a. Marked defect in cellular response to chemotaxis
   b. Failure to exhibit increased anaerobic metabolism during phagocytosis
   c. Mild to marked defect in bactericidal ability of neutrophils
   d. Defective leukocyte locomotion

29. Which statement about eosinophils is false?
   a. They are homeostatic regulators of inflammation.
   b. They attempt to suppress an inflammatory reaction.
   c. They participate in hypersensitivity reactions.
   d. They interact with the larval stages of some helminth parasites.

30. Which statement about basophils is false?
    a. They have a high concentration of heparin in the granules.
    b. They have a high concentration of histamine in the granules.
    c. They react with two adjacent IgA molecules on mast cells.
    d. They are associated with anaphylactic shock.

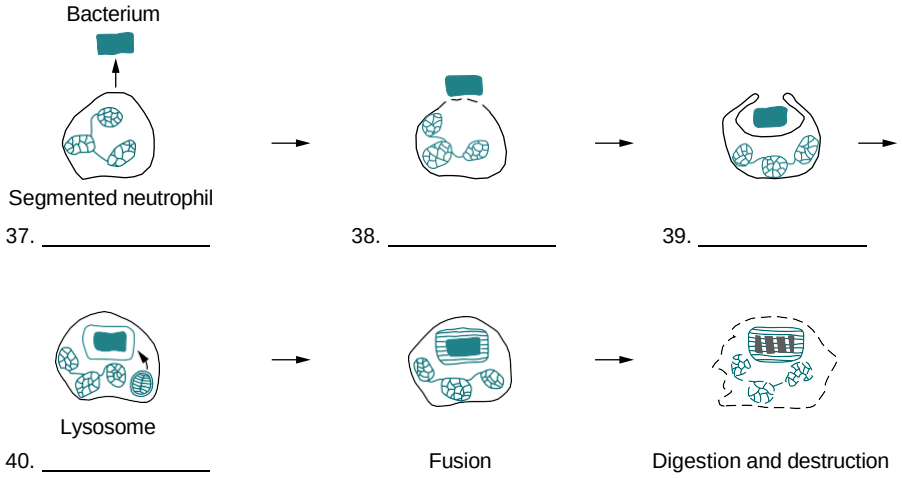
31. The cells that constitute the physiologic, mononuclear phagocyte system do not include:
    a. Promonocytes and their precursors
    b. Monocytes in circulating blood
    c. Macrophages in tissues
    d. Polymorphonuclear neutrophils

32-36. Identify the types of mononuclear phagocytic cells found in the various locations shown in the illustration. Choose from the following answers:
    a. Kupffer cells
    b. Macrophages
    c. Microglial cells
    d. Histiocytes (tissue macrophages)



(From Turgeon ML: Clinical hematology: theory and procedures, ed 5, Philadelphia, 2012, Lippincott Williams & Wilkins)

37-40. Name the steps in the process of phagocytosis shown in the illustration. Choose from the following answers:
    a. Engulfment
    b. Chemotaxis
    c. Phagosome formation
    d. Adherence



(From Turgeon ML: Clinical hematology: theory and procedures, ed 5, Philadelphia, 2012, Lippincott Williams & Wilkins)

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CHAPTER 4

Cells and Cellular Activities of the Immune System: Lymphocytes and Plasma Cells

Lymphocytes and Plasma Cells
Lymphoid and Nonlymphoid Surface Membrane Markers
Sites of Lymphocyte Development
Circulation of Lymphocytes
Virgin or Naïve Lymphocytes
Development of T Lymphocytes
Early Cellular Differentiation and Development
Antigen Processing and Antigen Presentation to T Cells
Antigen Recognition by T Cells
T-Independent Antigen Triggering
Natural Killer and K-Type Lymphocytes
Natural Killer Cells
K-Type Lymphocytes
Development and Differentiation of B Lymphocytes
B Lymphocyte Subsets
Cell Surface Markers
B Cell Activation
Plasma Cell Biology
Alterations in Lymphocyte Subsets
Changes With Aging
Evaluation of Immunodeficiency Syndromes
Cell-Mediated Immune System
Humoral System
Immunologic Disorders
Primary Immunodeficiency Disorders
Secondary Immunodeficiency Disorders
Immune-Mediated Disease
Autoimmune Lymphoproliferative Syndrome
Case Study
Questions
Critical Thinking Group Discussion Questions
Procedure: Assessment of Cellular Immune Status
Procedure: Results
Chapter Highlights
Review Questions
Bibliography

Learning Objectives

At the conclusion of this chapter, the reader should be able to:

- Differentiate and compare the function of primary and secondary lymphoid tissues.
- Describe the structure and function of a lymph node.
- Explain the role of the thymus in T lymphocyte maturation.
- Describe the maturation of a B lymphocyte from origination to plasma cell development.
- Compare the function of T lymphocytes and B lymphocytes in immunity.
- Explain the function of natural killer (NK) cells.
- Define the term *cluster of differentiation* (CD) and explain the purpose of detecting this marker.
- Differentiate the characteristics of T lymphocyte subsets on the basis of antigen structures and function.
- Describe the evaluation of suspected lymphocytic or plasma cell defects.
- Name and compare disorders of immunologic (lymphocytic or plasma cell) origin.
- Compare various categories of immunodeficiency disorders.
- Analyze and apply knowledge from this chapter to a representative case study.
- Correctly answer case study–related multiple choice questions.
- Be prepared to participate in a discussion of critical thinking questions.
- Describe the assessment of the cellular immune status.
- Correctly answer end of chapter review questions.

Key Terms

allograft
anergy
antigen-presenting cells (APCs)
B lymphocytes
cell surface markers
cluster of differentiation (CD)
cytotoxic
double-negative lymphocytes
double-negative thymocytes
double-positive thymocytes
dyscrasias
dysplastic
effector T cells
endogenous pathway
exogenous pathway
granzyme A-B
gut-associated lymphoid tissue (GALT)
immune senescence
immunodeficiency syndromes
immunoproliferative
immunoregulatory cells
immunosuppression
interleukin (IL)
lymphocyte recirculation
macrophages
memory cells
monoclonal gammopathies
monocytes
natural killer cells
natural Treg cells
negative selection
neoantigen
plasma cells
positive selection
primary immunodeficiency disorders
suppressor-cytotoxic lymphocytes
surface immunoglobulin (sIg)
T cell receptor (TCR)
T lymphocytes
thymus
T-independent antigens

LYMPHOCYTES AND PLASMA CELLS

The adaptive immune system is comprised of the humoral and cellular systems. Each of the two arms of the adaptive immune system has fundamental mechanisms allowing the body to attack an invading pathogen. The immunologically specific cellular component of the immune system is organized around two classes of specialized cells, **T lymphocytes** and **B lymphocytes.** Lymphocytes recognize foreign antigens, directly destroy some cells, or produce antibodies as **plasma cells.** The total immune response involves the interaction of many different cell types and cell-mediated and antibody-mediated responses. Recent studies have shown that T cells are not just the latecomers in inflammation, but might also play a key role in the early phase of this response. T cell subsets, including NK cells, together with classic innate immune cells, contribute significantly to the development and establishment of acute and chronic inflammatory diseases.

LYMPHOID AND NONLYMPHOID SURFACE MEMBRANE MARKERS

Before 1979, human lymphocytes could be classified as T or B cells based on observation of these cells with electron microscopy (Fig. 4-1). T lymphocytes have a relatively smooth surface compared with the rough pattern of the B lymphocytes.

The introduction of monoclonal antibody (MAb) testing (see Chapter 2) led to the present identification of surface membrane markers on lymphocytes and other cells. In practical terms, surface markers are used to identify and enumerate various lymphocyte subsets, establish lymphocyte maturity, classify leukemias, and monitor patients on immunosuppressive therapy.

Cell surface molecules recognized by MAbs are called antigens, because antibodies can be produced against them, or markers, because they identify and discriminate between, or "mark," different cell populations. Originally, surface markers

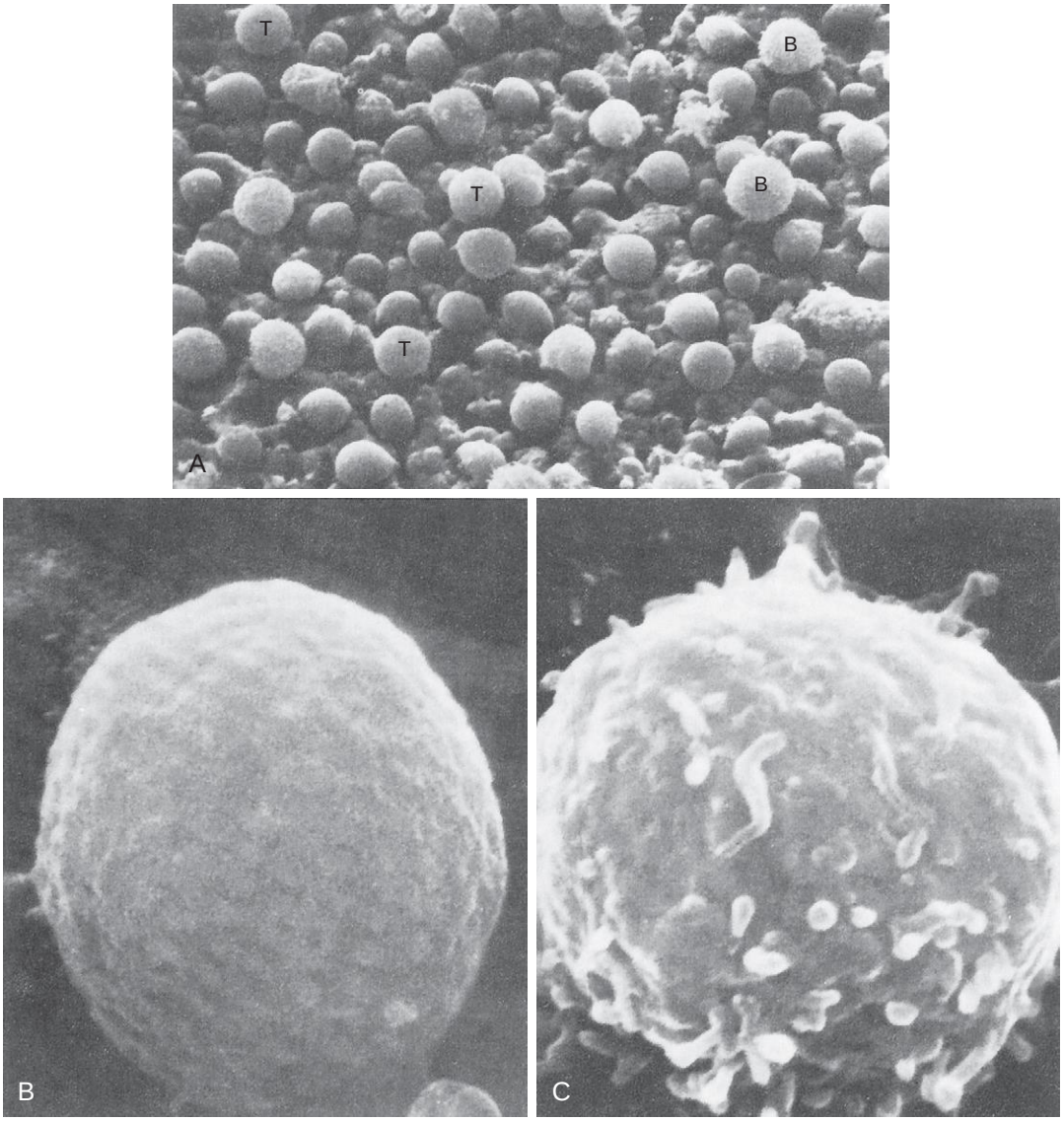


Figure 4-1 Scanning electron photomicrographs of lymphocyte cell surface membranes. **A**, T and B lymphocytes. **B**, T lymphocyte. **C**, B lymphocyte. *(From Polliack A, Lampen N, Clarkson BD, et al: Identification of human B and T lymphocytes by scanning electron microscopy, J Exp Med 138:607–624, 1973.)*

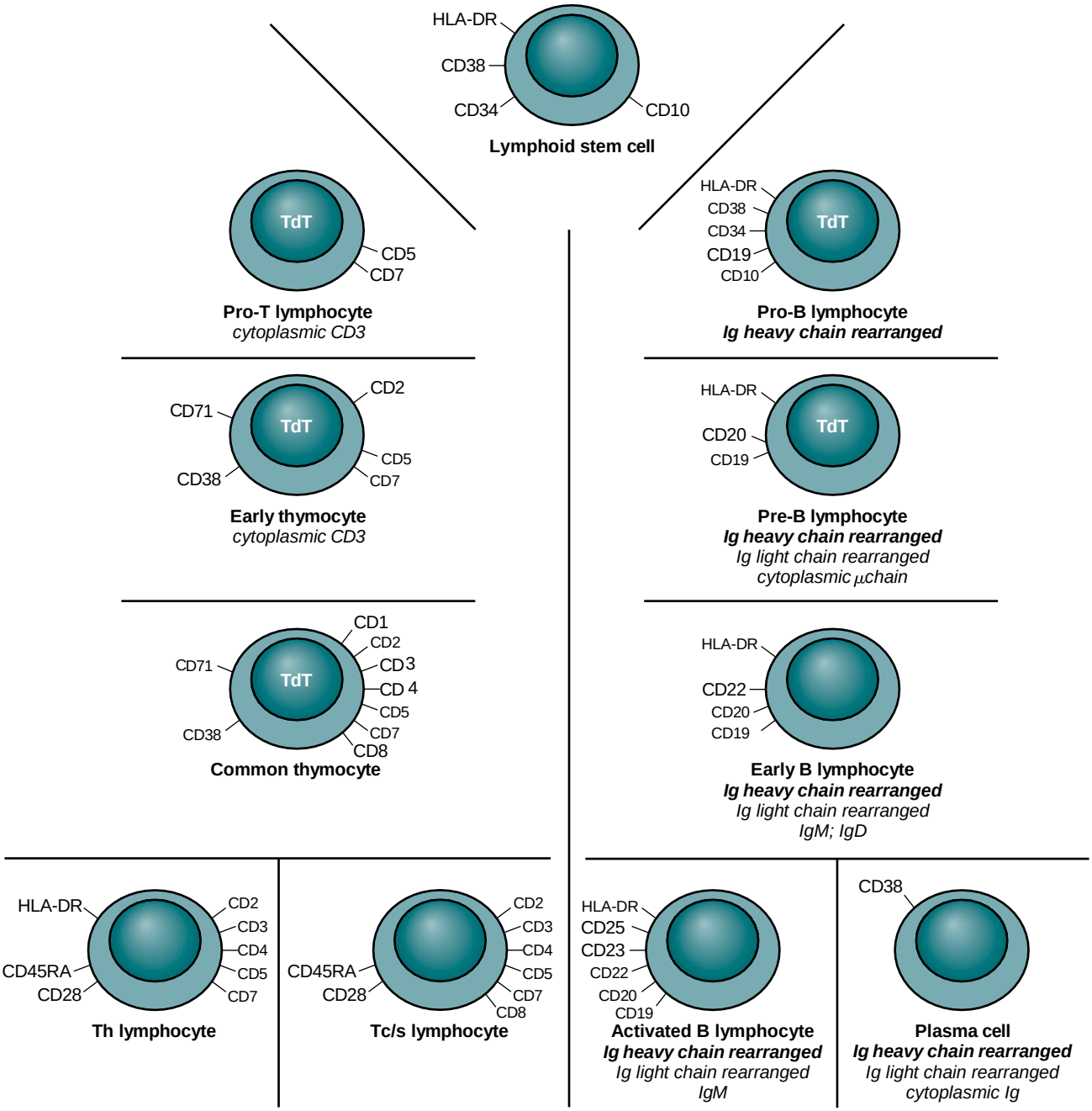


Figure 4-2 Lymphocyte membrane marker development—lymphoid CD antigen expression in T lymphocytes. *TdT*, Terminal deoxynucleotidyl transferase. *CD*, cluster of differentiation; *HLA*, human leukocyte antigen; *Ig*, immunoglobulin.

were named according to the antibodies that reacted with them, but a uniform nomenclature system has now been adopted.

In this system, a surface marker that identifies a particular lineage or differentiation stage with a defined structure, and that can be identified with a group or cluster of MAbs, is called a member of a **cluster of differentiation** (**CD**; Fig. 4-2). Markers can be categorized as follows:

- Some markers are specific for cells of a particular lineage or maturational pathway.
- Some markers vary in expression, depending on the state of activation or differentiation of the same cells—for example, when CD antigen identification is used to classify lymphocyte subsets (e.g., CD4 and CD8).

In addition to using CD classification for the identification and separation of lymphocytes, CD antigens are involved in various lymphocyte functions, usually the following:

- Promotion of cell to cell interactions and adhesion
- Transduction of signals that lead to lymphocyte activation

Sites of Lymphocyte Development

In mammalian immunologic development, the precursors of lymphocytes arise from progenitor cells of the yolk sac and liver (Fig. 4-3). Later in fetal development, and throughout the life cycle, the bone marrow becomes the sole provider of undifferentiated progenitor cells, which can further develop into lymphoblasts. Continued cellular development and proliferation of

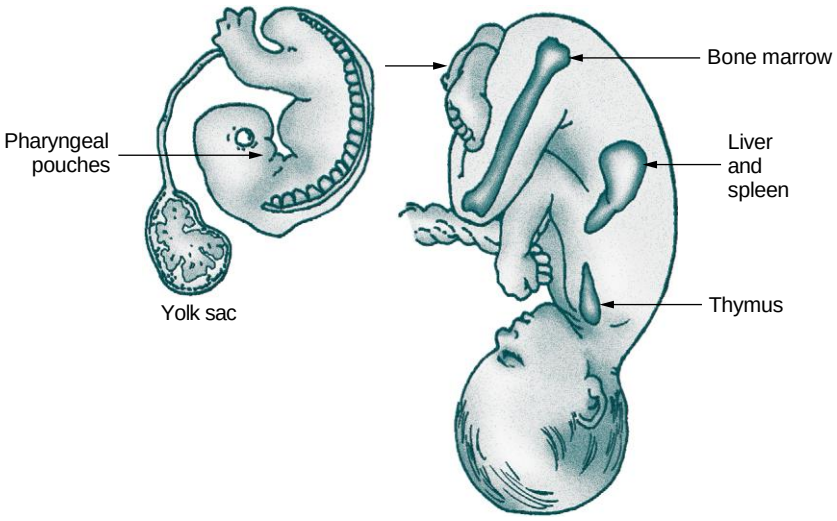


Figure 4-3 Development of immunologic organs. The anatomy of the human fetus illustrates the development of the mammalian immune system. Cells of the pharyngeal pouches migrate into the chest and form the thymus. Precursors of lymphocytes originate early in embryonic life in the yolk sac and eventually migrate to the bone marrow via the spleen and liver.

lymphoid precursors occur as the cells travel to the primary and secondary lymphoid tissues.

Primary Lymphoid Tissue

In mammals, both the bone marrow (and/or fetal liver) and thymus are classified as primary or central lymphoid organs (Fig.4-4).

Thymus. Early in embryonic development, the stroma and nonlymphoid epithelium of the **thymus** are derived from the third and fourth pharyngeal pouches. The characteristics of the thymus gland change with aging. Older persons are immunologically challenged because aging causes a reduction in the production of naïve T cells by the thymus. Intrinsic defects in mature T cell function, alterations in the life span of naïve T cells and in naïve or memory T cell ratios in the peripheral lymphoid tissues, occur as the result of the decline of the T cell response in older persons.

The thymus, located in the mediastinum, exercises control over the entire immune system. It is believed that the development of diversity occurs mainly in the thymus and bone marrow, although clonal expansion can occur anywhere in the peripheral lymphoid tissue.

Progenitor cells that migrate to the thymus proliferate and differentiate under the influence of the humoral factor, thymosin. These lymphocyte precursors with acquired surface membrane antigens are referred to as thymocytes.

The reticular structure of the thymus allows a significant number of lymphocytes to pass through it to become fully immunocompetent (able to function in the immune response), thymus-derived T cells. The thymus also regulates immune function by the secretion of multiple soluble hormones.

Many cells die in the thymus and apparently are phagocytized, a mechanism to eliminate lymphocyte clones reactive against self. It is estimated that approximately 97% of the cortical cells die in the thymus before becoming mature T cells. Viable cells migrate to the secondary tissues. The absence or abnormal development of the thymus results in a T lymphocyte deficiency.

Involution of the thymus is the first age-related change occurring in the human immune system. In postnatal life, the thymus is the primary organ that produces naïve T cells for the peripheral T cell pool but production of cells declines as early as 3 months of age. The thymus gradually loses up to 95% of its mass during the first 50 years of life (Fig. 4-5). The accompanying functional changes of decreased synthesis of thymic hormones and the loss of ability to differentiate immature lymphocytes are reflected in an increased number of immature lymphocytes within the thymus and as circulating peripheral blood T cells. Most changes in immune function, such as dysfunction of T and B lymphocytes, elevated levels of circulating immune complexes, increases in autoantibodies, and **monoclonal gammopathies** are correlated with involution of the thymus (see Chapter 27). **Immune senescence** may account for the increased susceptibility of older adults to infections, autoimmune disease, and neoplasms.

Bone Marrow. The bone marrow is the source of progenitor cells. These cells can differentiate into lymphocytes and other hematopoietic cells (e.g., granulocytes, erythrocytes, megakaryocyte populations). In mammals, the bone marrow also supports eventual differentiation of mature T and B lymphocytes, probably from a common lymphoid cell progenitor. It is believed that the bone marrow and **gut-associated lymphoid tissue (GALT)** may also play a role in the differentiation of progenitor cells into B lymphocytes.

Secondary Lymphoid Organs

Secondary lymphoid organs provide a unique microenvironment for the initiation and development of immune responses. The secondary lymphoid tissues include lymph nodes, spleen, GALT, thoracic duct, bronchus-associated lymphoid tissue (BALT), skin-associated lymphoid tissue, and blood. Mature lymphocytes and accessory cells (e.g., antigen-presenting cells) are found throughout the body, although the relative percentages of T and B cells vary in different locations (Table 4-1).

The highly sophisticated structure of secondary lymphoid organs allows migration and interactions between antigen-presenting cells, T and B lymphocytes, and follicular dendritic cells (FDCs) and other stromal cells. The cooperative activities of lymphoid cells within secondary organs dramatically increase

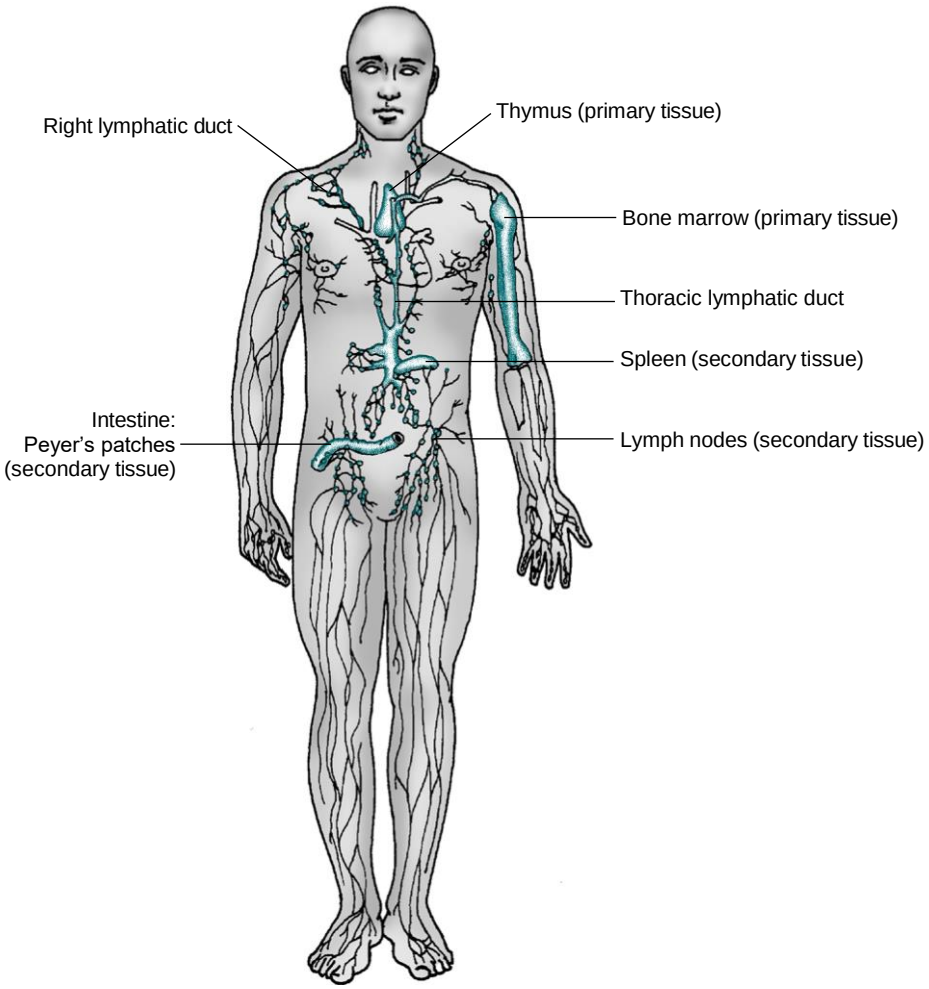


Figure 4-4 Human primary and secondary tissues. *(Adapted from Turgeon ML: Clinical hematology: theory and procedures, ed 4, Philadelphia, 2005, Lippincott Williams & Wilkins.)*

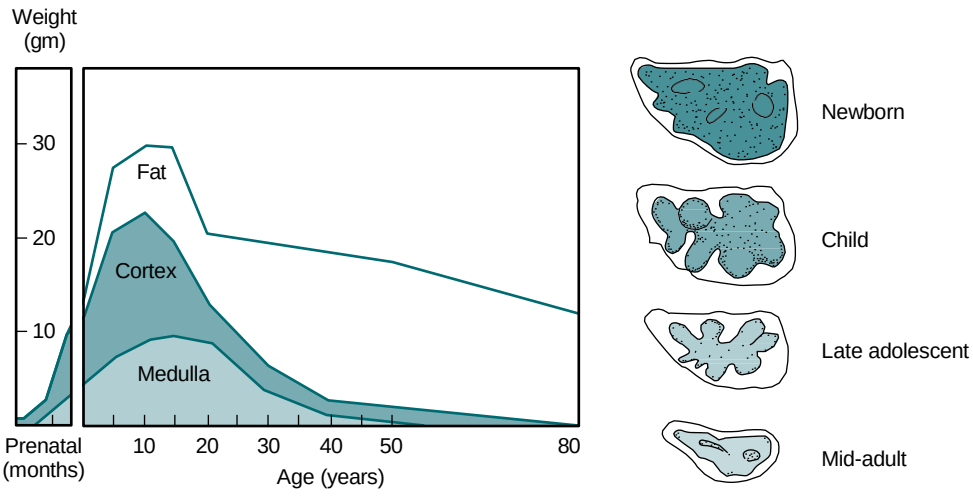


Figure 4-5 Thymic development. Histology of the thymus changes with age. The main feature of these changes is a loss of cellularity with increasing age.

the probability of interactions of rare B, T, and APCs that results in effective generation of humoral immune responses.

Tumor necrosis factor (TNF) and lymphotoxin are essential to the formation and maintenance of secondary organs. These cytokines are produced by B and T lymphocytes. Proliferation of the T and B lymphocytes in the secondary or peripheral lymphoid tissues (Fig. 4-6) is primarily dependent on antigenic stimulation.

The T lymphocytes or T cells populate the following:

1. Perifollicular and paracortical regions of the lymph nodes
2. Medullary cords of the lymph nodes

Table 4-1 Approximate Percentage of Lymphocytes in Lymphoid Organs

Lymphoid Organ	T Lymphocytes (%)	B Lymphocytes (%)
Thymus	100	0
Blood	80	20
Lymph nodes	60	40
Spleen	45	55
Bone marrow	10	90

Adapted from Claman HN: The biology of the immune response, JAMA 268:2790-2796, 1992.

3. Periarteriolar regions of the spleen
4. Thoracic duct of the circulatory system

The B lymphocytes or B cells multiply and populate the following:

1. Follicular and medullary (germinal centers) of the lymph nodes
2. Primary follicles and red pulp of the spleen
3. Follicular regions of GALT
4. Medullary cords of the lymph nodes

Lymph Nodes. Lymph nodes act as lymphoid filters in the lymphatic system. Lymph nodes respond to antigens introduced distally and routed to them by afferent lymphatics (Fig. 4-7). Generalized lymph node reactivity can occur after systemic antigen challenge (e.g., serum sickness).

Spleen. The spleen acts as a lymphatic filter within the blood vascular tree. It is an important site of antibody production in response to IV particulate antigens (e.g., bacteria). The spleen is also a major organ for the clearance particles.

Gut-Associated Lymphoid Tissue. GALT includes lymphoid tissue in the intestines (Peyer's patches) and the liver. GALT features immunoglobulin A (IgA) production and involves a unique pattern of **lymphocyte recirculation.** Pre–B cells develop in Peyer's patches and, after meeting antigen from the gut, many enter the general circulation and then return back to the gut. GALT is also important for the development of tolerance to ingested antigens.

Thoracic Duct. The thoracic duct lymph is a rich source of mature T cells. Chronic thoracic duct drainage can cause T cell depletion and has been used as a method of **immunosuppression.**

Bronchus-Associated Lymphoid Tissue. BALT includes lymphoid tissue in the lower respiratory tract and hilar lymph nodes. It is mainly associated with IgA production in response to inhaled antigens.

Skin-Associated Lymphoid Tissue. Antigens introduced through the skin are presented by epidermal Langerhans cells, which are bone marrow–derived accessory cells. These epidermal cells then interact with lymphocytes in the skin and in draining lymph nodes.

Blood. The blood is an important lymphoid organ and immunologic effector tissue. Circulating blood has enough mature T cells to produce a graft-versus-host reaction. In addition, blood transfusions have been responsible for inducing acquired immunologic tolerance in kidney **allograft** patients.

Blood is the most frequently sampled lymphoid organ. It is assumed that what is found in blood samples represents what is present in other lymphoid tissues. Although this may be a true representation, it is not always accurate.

Circulation of Lymphocytes

Mature T lymphocytes survive for several months or years, whereas the average life span of B lymphocytes is only a few days. Lymphocytes move freely between the blood and lymphoid tissues. This activity, termed *lymphocyte recirculation,* enables lymphocytes to come into contact with processed foreign antigens and disseminate antigen-sensitized memory cells throughout the lymphoid system. Clonal expansion may occur regionally, as in lymph nodes draining a contact allergic reaction, and then the whole body becomes susceptible to rechallenge because T cells recirculate, but generally are excluded from returning to the thymus. Research has shown that a pool of T cell clonal elements is developed by a combination of **positive selection** of clones able to recognize and react to foreign antigens, and **negative selection** (purging) of clones able to interact with self-antigens in a damaging way.

Recirculation of lymphocytes back to the blood is through the major lymphatic ducts. Lymphocytes enter the lymph node from the blood circulation via arterioles and capillaries to reach the specialized postcapillary venules. From the venule, the lymphocytes enter the node and remain in the node or pass through the node and return to the circulating blood. Lymphatic fluid, lymphocytes, and antigens from certain body sites enter the lymph node through the afferent lymphatic duct and exit the lymph node through the efferent lymphatic duct (see Fig. 4-7).

VIRGIN OR NAÏVE LYMPHOCYTES

Virgin or naïve lymphocytes are cells that have not encountered their specific antigen. These cells do express high-molecular-weight variants of leukocyte common antigen.

Memory cells are populations of long-lived T or B cells that have been stimulated by antigen. They can make a quick response to a previously encountered antigen. Memory B cells carry surface IgG as their antigen receptor; memory T cells express the CD45RO variant of the leukocyte common antigen and increased levels of cell-adhesion molecules (CAMs), chemical mediators involved in inflammatory processes throughout the body (Fig. 4-8).

DEVELOPMENT OF T LYMPHOCYTES

Most lymphocytes (see Color Plate 8) found in the circulating blood are T cells derived from bone marrow progenitor cells that mature in the thymus gland (Table 4-2). These cells are responsible for cellular immune responses and are involved in the regulation of antibody reactions in conjunction with B lymphocytes.

During cellular development, T lymphocyte function–associated antigens vary in expression. Some antigens appear

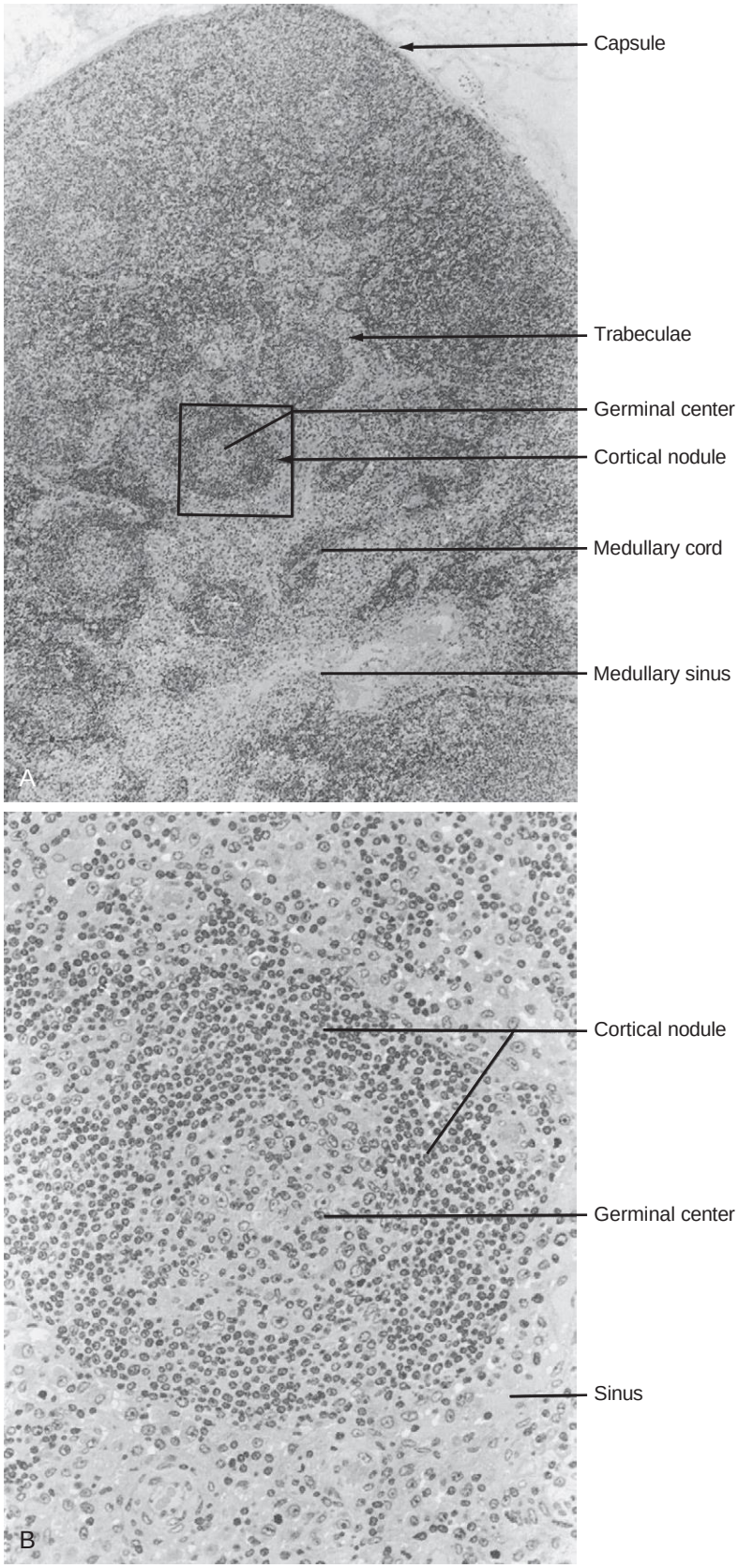


Figure 4-6 **A**, Lymph node (×90). **B**, Enlargement of cortical nodule seen in **A** (×450). *(From Anthony CP, Thibodeau GA: Textbook of anatomy and physiology, ed 12, St Louis, 1987, Mosby.)*

early in cellular development and remain on mature T cells. Others appear at an early or intermediate stage of cellular maturation and are lost before maturity.

Early Cellular Differentiation and Development

Differentiation of a lymphocyte begins in the thymus as a thymocyte. Early surface markers on thymocytes that are committed to becoming T cells include CD44 and CD25. As thymocytes develop, there is an orderly rearrangement of the genes coding for an antigen receptor.

Maturation is a complicated process that lasts for a period of 3 weeks. During this period, cells filter through the cortex to the medulla of the thymus. Thymic stromal cells include fibroblasts, macrophages, epithelial cells, and dendritic cells; all these cell types play a role in T cell development.

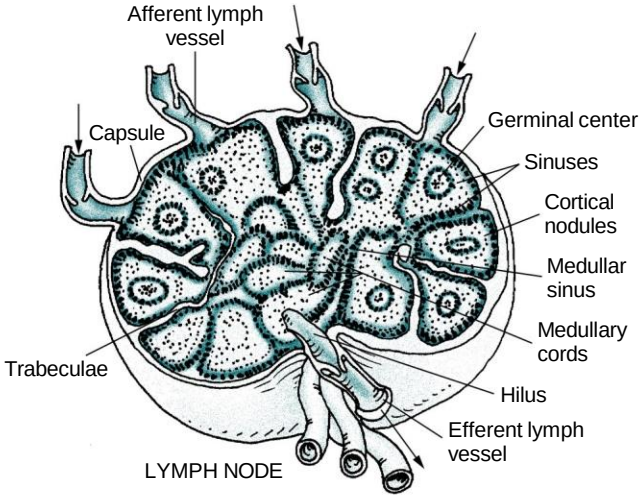


Figure 4-7 Structure of a lymph node. Several valved afferent lymphatics bring lymph to the node. An efferent lymphatic leaves the node at the hilus. Note that the artery and vein enter and leave at the hilus. *(Adapted from Anthony CP, Thibodeau GA: Textbook of anatomy and physiology, ed 12, St Louis, 1987, Mosby.)*

Double-Negative Thymocytes

Early thymocytes lacking CD4 and CD8 surface membrane markers are referred to as **double-negative thymocytes.** These cells proliferate in the outer cortex of the thymus under the influence of interleukin-7 (IL7). IL-7 is critical for this growth and differentiation.

Rearrangement of the genes that code for the antigen receptor, the **T cell receptor (TCR),** begin at this developmental stage. CD3 constitutes the main part of the T cell antigen receptor. The configuration of two of the eight chains of the receptor have variable regions that recognize specific antigens. These are coded for by selecting gene segments and deleting others.

Rearrangement of the beta (β) chain occurs first; the appearance of a functional β chain on the cell surface sends a signal to suppress any further β chain gene rearrangements.The combination of the β chain with the CD3 forms the pre– T-cell antigen receptor (TRC). Signaling by the β chain promotes the development of a CD4+ and CD8+ thymocyte.

Thymocytes that express gamma (γ) and delta (δ) chains follow a different developmental pathway. Cells expressing gamma-delta ($\gamma\delta$) chains typically remain both CD4– and CD8–. These double-negative cells represent most of the population of T lymphocytes in the skin and intestinal and pulmonary epithelium.

Circulating CD3+ **double-negative lymphocytes** are phenotypically and functionally distinct from single-positive CD3+CD4+ and CD3+CD8+ lymphocytes and are thought to represent a distinct T cell lineage. The presence of low numbers

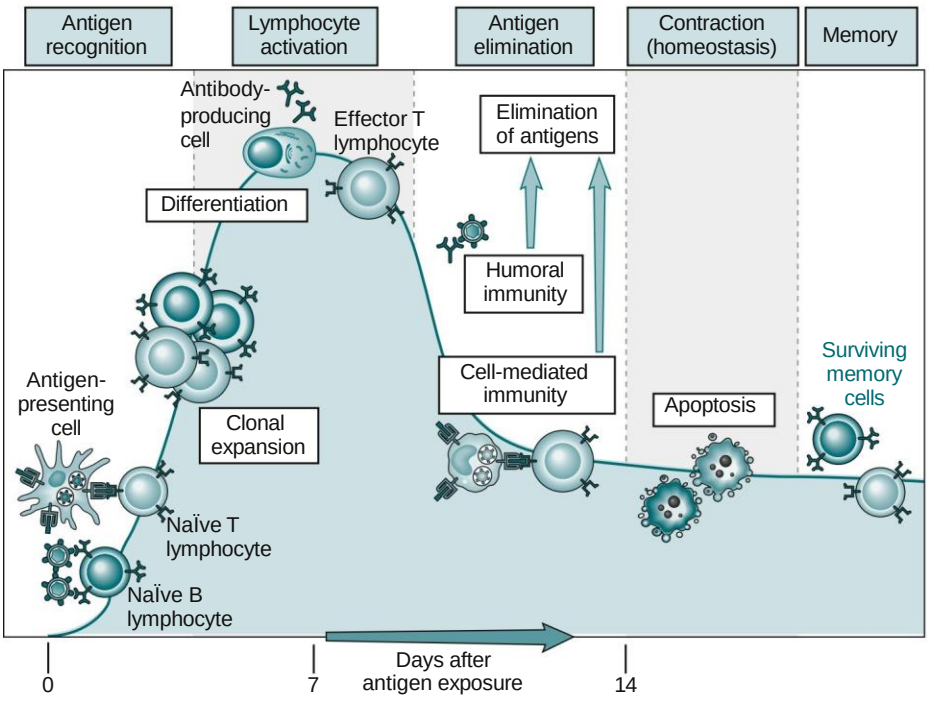


Figure 4-8 Phases of an adaptive immune response. An adaptive immune response consists of distinct phases; the first three are the recognition of antigen, activation of lymphocytes, and elimination of antigen (effector phase). The response declines as antigen-stimulated lymphocytes die by apoptosis, restoring homeostasis, and the antigen-specific cells that survive are responsible for memory. The duration of each phase may vary in different immune responses. The y-axis represents an arbitrary measure of the magnitude of the response. These principles apply to humoral immunity (measured by B lymphocytes) and cell-mediated immunity (mediated by T lymphocytes). *(From Abbas AK, Lichtman AH: Basic immunology: functions and disorders of the immune system, updated edition, ed 3, Philadelphia, 2011, Saunders.)*

Table 4-2 Lymphocyte Characteristics

Type	Function(s)	Phenotypic Marker	Peripheral Blood (% of Total)
Helper T (Th) cells	Stimulate B cell growth and differentiation (humoral immunity); macrophage activation by secreted cytokines (cell-mediated immunity)	CD3+, CD4+, CD8−	50-60
Cytotoxic T (Tc) cells	Lysis of virus-infected cells, tumor cells, and allografts (cell-mediated immunity); macrophage activation by secreted cytokines (cell-mediated immunity)	CD3+, CD4−, CD8+	20-25
Natural killer (NK) cells	Lysis of virus-infected cells, (antibody-dependent cellular cytotoxicity)	Fc receptor for IgG or cells CD16	~10
B cells	Antibody production (humoral immunity)	Fc receptors, MHC class II, CD19, CD21	10-15

of double-negative T cells in healthy individuals and the increase observed in association with lymphoproliferative disorders, graft-versus-host disease, and autoimmune diseases suggest a pathogenic or immunoregulatory role for this population of T lymphocytes.

Double-Positive Thymocytes

Cells with both CD4+ and CD8+, or double-positive, surface markers represent the second stage of thymocyte development. These thymocytes begin to demonstrate rearranged genes coding for the alpha (α) chain. When the CD3-$\alpha\beta$ receptor complex (TCR) is expressed on the cell surface, a process known as positive selection permits only double-positive cells with functional TCR receptors to survive. T cells must recognize foreign antigen in association with class I or II major histocompatibility complex (MHC) molecules. Any thymocyte that is unable to recognize self-MHC dies without ever leaving the thymus gland. Functioning T lymphocytes must be able to recognize a foreign antigen along with MHC molecules. A second selection process, negative selection, takes place among the surviving double-positive T cells. Only 1% to 3% of **double-positive thymocytes** survive in the cortex.

Double-positive (DP) CD4CD8 T$\alpha\beta$ cells have been reported in normal individuals as well as in different pathologic conditions, including inflammatory diseases, viral infections and cancer, but their function remains to be elucidated. Double-negative cells may act like natural killer (NK) cells because they are capable of binding to many natural, unprocessed cell surface molecules. In addition, these cells are capable of recognizing antigens without being presented by MHC proteins. Consequently, NK cells may represent an important bridge between natural and adaptive immunity.

Later Cellular Differentiation and Development of T Lymphocytes

When mature T cells leave the thymus, their T cell receptors (TCRs) are CD4+ or CD8+. Survivors of selection exhibit only one type of marker, CD4+ or CD8+ and migrate to the medulla. These cells gain functional maturity with their entry into the peripheral blood circulation.

T cells develop into a variety of clones. Each lymphocyte displays a single type of structurally unique receptor. The repertoire of antigen receptors in the entire population of lymphocytes is extremely large and diverse. This increases the probability that an individual lymphocyte will encounter an antigen that binds to its receptor, thereby triggering activation and proliferation of the cell. This process, clonal selection, accounts for most of the basic properties of the adaptive immune system.

Antigen receptors for common pathogens need to be reinvented by every generation of cells. Because the binding sites of antigen receptors arise from random genetic mechanisms, the receptor repertoire contains binding sites that can react not only with infectious microorganisms, but also with innocuous environmental antigens and self antigens.

T-Lymphocyte Subsets

The CD4+ subset was initially described as representing the helper-inducer T cell; the CD8+ subset was initially described as representing the suppressor-cytotoxic T cell. Lymphocytes can be subdivided into several populations using various operational and phenotypic parameters. For example, CD4 lymphocytes express both CD3 and CD4. The surface marker CD45RA subset delineates a naïve helper T cell population. The CD45RO subset delineates a memory helper T cell population. CD8+ lymphocytes express both CD3 and CD8 surface membrane markers.

Helper T Lymphocytes

Helper T lymphocytes, or T-helper (Th) cells, can be assigned to one of several subsets, including the following:

- Helper T type 1 (Th1) cells are responsible for cell-mediated effector mechanisms.
- Helper T type 2 (Th2) cells play a greater role in the regulation of antibody production.
- Regulatory T (Treg) cells are an immunoregulatory type of Th cells.

These divisions are not absolute, with considerable overlap or redundancy in function among the different subsets. This classification is based on the in vitro blends of cytokines that

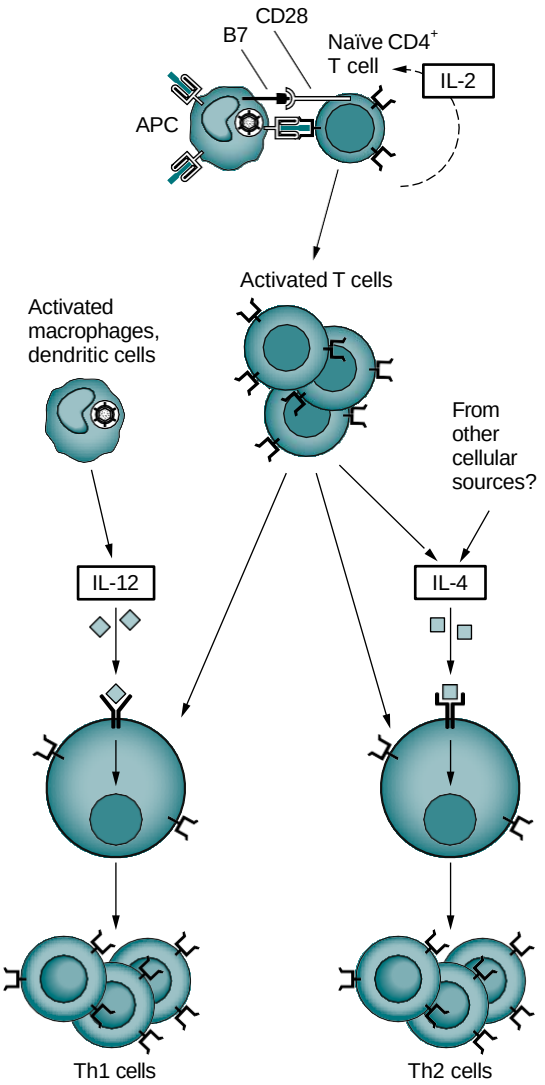


Figure 4-9 Differentiation of naïve CD4+ helper T (Th) cells into Th1 and Th2 effector cells. After their activation by antigen and costimulators, naïve helper T cells may differentiate into Th1 and Th2 cells under the influence of cytokines. IL-12 produced by microbe-activated macrophages and dendritic cells stimulates differentiation of CD4+ T cells into Th1 effectors. In the absence of IL-12, the T cells themselves (and perhaps other cells) produce IL-4, which stimulates their differentiation into Th2 effectors. *(Adapted from Abbas AK, Lichtman AH: Basic immunology: functions and disorders of the immune system, ed 3, Philadelphia, 2008, Saunders.)*

they produce. Th1 and Th2 cells can promote the development of **cytotoxic** cells and are believed to develop from Th0 cells. Th1 cells interact most effectively with mononuclear phagocytes; Th2 cells release cytokines that are required for B cell differentiation (Fig. 4-9).

Characterized by high interferon-gamma (IFN-γ) production, Th1 responses promote the elimination of intracellular pathogens (Fig. 4-10, *A*). Characterized by IL-4 and IL-5, Th2 responses promote a different type of effector response that involves immunoglobulin E (IgE) production and eosinophils capable of eliminating larger extracellular pathogens, such as helminths (see Fig. 4-10, *B*). In situations of repeated pathogen exposure or persistent infection, the polarization of T cell responses serves to focus the antigen-specific response on a specific effector pathway.

The following factors can influence the terminal differentiation of lymphocytes:

- Type of antigen-presenting cell (APC)
- Affinity of the specific antigenic peptide
- Types of costimulatory molecules expressed by APCs
- Cytokines acting on T cells during primary activation through TCRs

A hierarchy is apparent among these factors and is determined by how they influence T cell differentiation. Certain cytokines acting directly on T cells during primary activation appear to be the most proximal or direct mediators of CD4+ T cell differentiation. The presence of IL-12 during primary T cell activation leads to strong development of Th1 responses, and IL-4 promotes Th2 development. Activation through the TCR is a requirement for initiating terminal differentiation, but the signals from the TCR appear to be phenotype-neutral.

Certain T cells carry out delayed hypersensitivity reactions. These T cells react with antigen MHC class II on APCs and create their effects mainly through cytokine production. These cells generally are of the CD4+ phenotype.

T cells can also be differentiated into two populations depending on whether they use an $\alpha\beta$ (TCR2) or $\gamma\delta$ (TCR1) antigen receptor. The TCR consists of a heterodimer and a number of associated polypeptides that form the CD3 complex. The dimer recognizes processed antigen associated with an MHC molecule. The CD3 complex is required for receptor expression and is involved in signal transduction. TCR1 cells constitute less than 5% of total lymphocytes but appear in greater proportions in some sites (e.g., skin, vagina). TCR1 cells appear to recognize different antigens than TCR2 cells, including carbohydrate and intact protein antigens. In addition, some TCR1 cells do not require antigen to be processed or presented by MHC molecules.

T Regulatory Lymphocytes

Treg cells are immunoregulatory Th cells that control autoimmunity in the peripheral blood through dominant tolerance. Types of Treg cells include the following:

- Natural CD4+ Treg cells
- Th3 cells
- Tr1 cells
- CD8+ Treg cells

Natural Treg cells, characterized by constitutive expression of CD25, are developed primarily in the thymus from positively selected thymocytes with a relatively high avidity for self antigens. Natural Treg cells represent approximately 5% to 10% of the total CD4+ T cell population. The signal to develop into Treg cells is thought to come from interactions between the TCR and MHC class II self-peptide complex expressed on the thymic stroma. In humans, natural Treg cells express CD4 and CD25.

Other types of Treg cells that can develop in the periphery are Tr1 and Th3 cells. Tr1 cells are CD4+ and are functionally

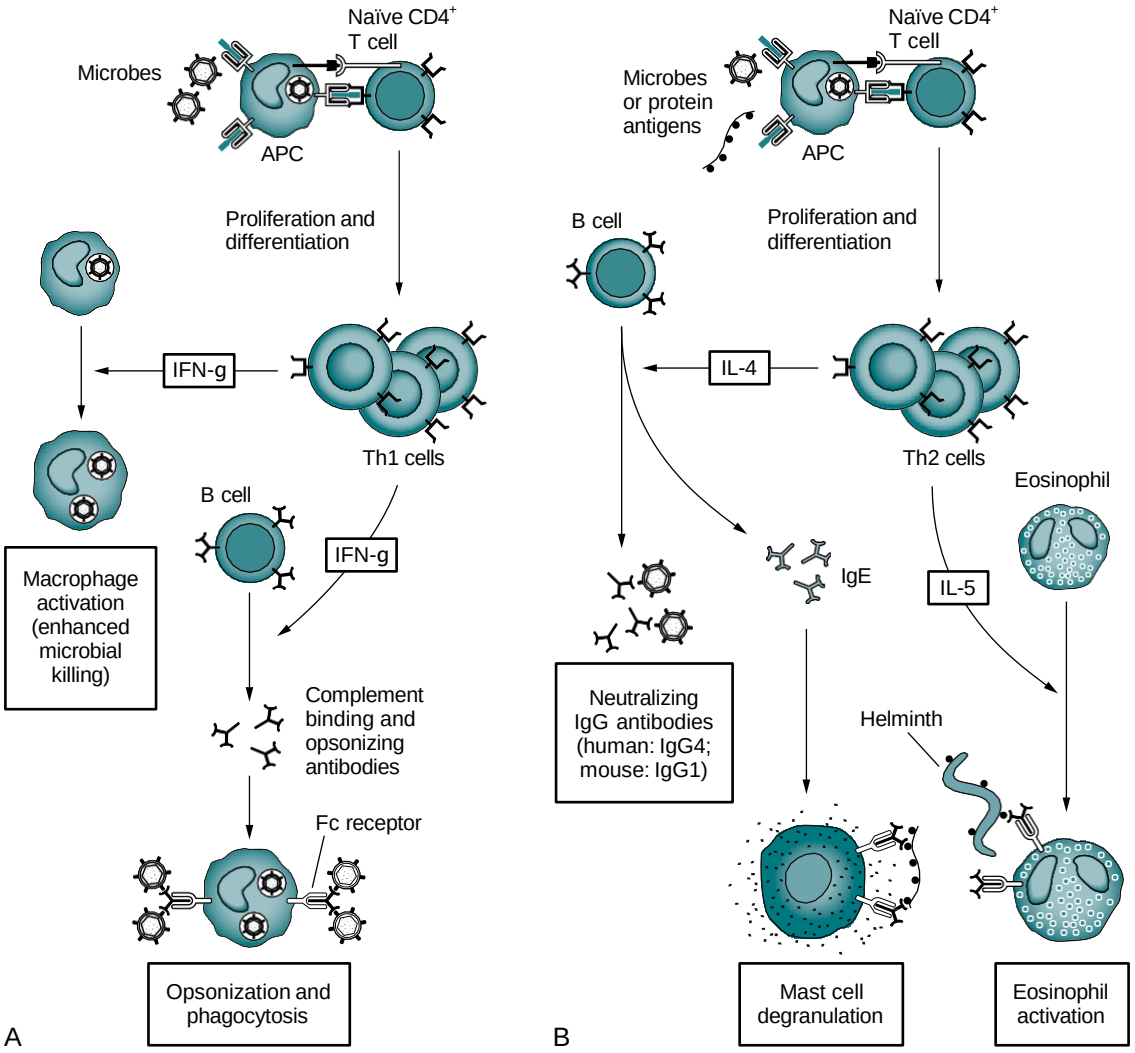


Figure 4-10 Functions of Th1 and Th2 subsets of CD4+ helper T lymphocytes. A, Th1 cells produce the cytokine IFN-γ, which activates phagocytes to kill ingested microbes and stimulates the production of antibodies that promote the ingestion of microbes by the phagocytes. **B,** Th2 cells specific for microbial or nonmicrobial protein antigens produce the cytokines IL-4, which stimulates the production of IgE antibody, and IL-5, which activates eosinophils. IgE participates in the activation of mast cells by protein antigens and coats helminths for destruction by eosinophils. Th2 cells also stimulate the production of other antibodies (IgG4 in humans) that neutralize microbes and toxins but do not bind to Fc receptors or activate complement efficiently. *(Adapted from Abbas AK, Lichtman AH: Basic immunology: functions and disorders of the immune system, ed 3, Philadelphia, 2008, Saunders.)*

induced by IL-10. These Treg cells, in turn, secrete IL-10 and regulate the immune system. Th3 progenitor cells are also CD4+. In vitro CD4+ cells have been shown to secrete transforming growth factor β (TGF-β). CD8+ Treg cells are less well characterized and are reportedly capable of suppressing CD4+ cells in vitro.

Cytotoxic T Lymphocytes

Cytotoxic T lymphocytes, or T cytotoxic (Tc) cells, are effector cells found in the peripheral blood that are capable of directly destroying virally infected target cells. Most Tc cells are CD8+ and recognize antigen on the target cell surface associated with MHC class I molecules (e.g., human leukocyte antigen [HLA] types A, B, and C) or MHC class I alone. This process is demonstrated by the immune response to virus-infected cells or tumor cells (Fig. 4-11).

In a primary viral infection, naïve CD8+ T cells are primed in secondary lymph nodes and consequently proliferate and differentiate into effector CD8+ T cells to eliminate virus-infected cells. After clearance of the virus, most effector CD8+ T cells contract because of apoptosis but a small number of these CD8+ T cells form a memory T cell pool.

Studies have demonstrated that human CD8+ T cells undergo a change in the expression of costimulatory molecules (e.g., CD27, CD28, and CD45RA) on their surface, according to their differentiation and maturation. Cytolytic effector molecules, perforin, and **granzyme A-B,** are considered to be markers for effector CD8+ T cells because they are the actual functional molecules for killing target cells.

Naïve and central memory CD8+ T cells express the membrane marker, CCR7, for homing to secondary lymph nodes, but effector memory and effector CD8+ T cells express the

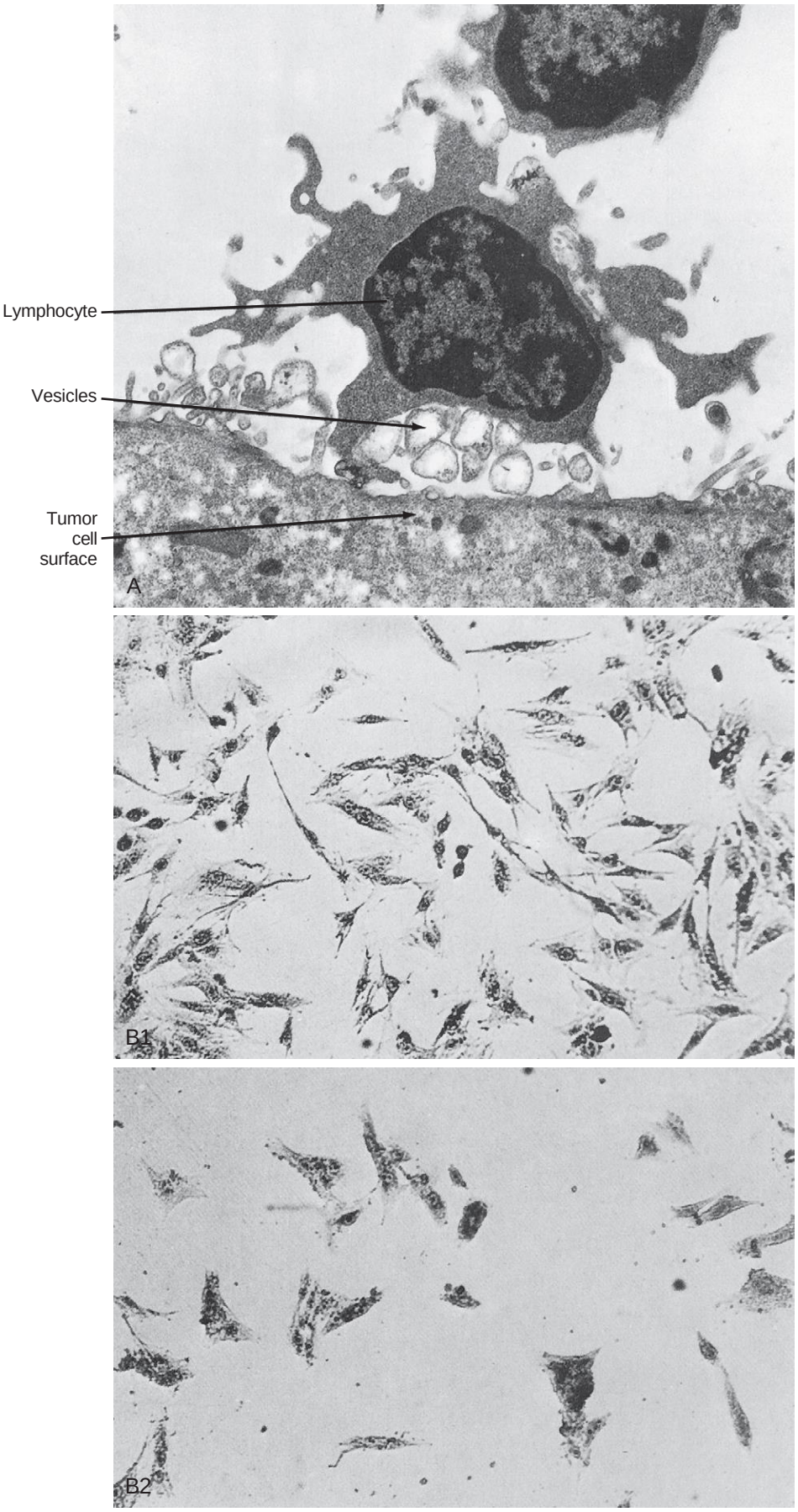


Figure 4-11 **A,** Transmission electron photomicrograph demonstrating the initial stages of the attack of a cytotoxic lymphocyte on a tumor cell, only a portion of which is seen. Note vesicles and blebs of cytoplasm being shed by the lymphocyte. **B,** Effects of cytotoxic lymphocytes on tumor cells. *1,* Tumor cells before contact with immune lymphocytes. *2,* Tumor cells after contact. Note that many cells have detached from the surface, some cells are swollen, and a few cells exhibit the morphology of normal cells. *(From Barrett JT: Textbook of immunology, ed 5, St Louis, 1988, Mosby.)*

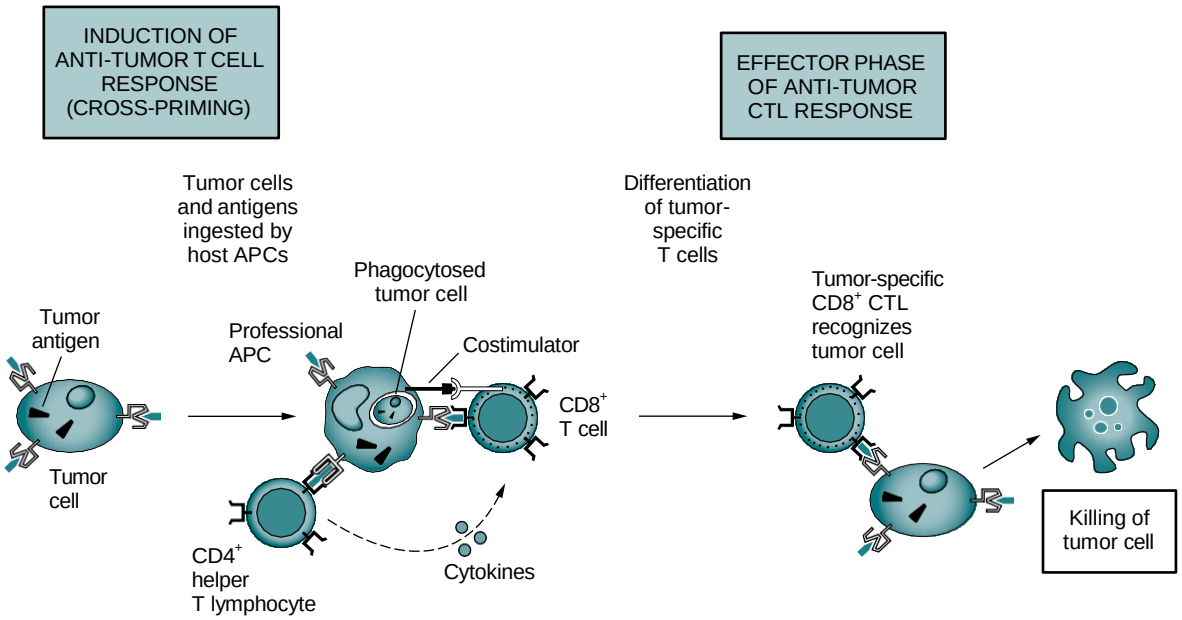


Figure 4-12 Induction of CD8+ T cell responses against tumors. Responses to tumors may be induced by cross-priming (or cross-presentation), in which the tumor cells and tumor antigens are taken up by specialized (so-called professional) APCs, processed, and presented to T cells. In some cases, B7 costimulators expressed by the APCs provide the second signals for the differentiation of the CD8+ T cells. APCs may also stimulate CD4+ helper T cells, which provide the second signals for cytotoxic T lymphocyte (CTL) development. Differentiated CTLs kill tumor cells without a requirement for costimulation or T cell assistance. *(From Abbas AK, Lichtman AH: Basic immunology: functions and disorders of the immune system, ed 3, Philadelphia, 2008, Saunders.)*

chemokine receptors for inflammatory cytokines, which enable the cells to migrate toward infected and inflamed sites. A unique subset of the effector CD8+ T cell population expresses CXCR1. These CXCR1 CD8+ T cells possess chemotactic activity toward the CDCR1 ligand IL-8, a potent inflammatory cytokine produced in inflamed tissues and in tissues infected with some viruses, such as human cytomegalovirus (HCMV) or influenza A. This suggests that these CXCR1+ effector CD8+ T cells immediately migrate to inflamed and infected sites to exert their effector function in the initial stage of an immune response. It is possible that effector CD8+ T cell subsets are functionally distinct populations of T lymphocytes.

In addition to destruction of virally infected, MHC class I–bearing targets, Tc cells are major effectors in allograft organ rejection. Tc cells express CD4 or CD8, depending on the MHC antigen restriction that governs their antigen recognition (i.e., class I or II antigens; Fig. 4-12).

Suppressor T lymphocytes, or T suppressor (Ts) cells, are functionally defined T cells that downregulate the actions of other T and B cells. Ts cells have no unique markers. Although antigen-specific suppression was described in 1970, and many investigators believe that Ts cells are critical in various phases of immunoregulation, peripheral tolerance, and autoimmunity, their mode of action is unclear. Many Ts cells are CD8+ and may operate through secretion of free TCRs.

Antigen Processing and Antigen Presentation to T Cells

Antigen-presenting cells (APCs) are a group of functionally defined cells capable of taking up antigens and presenting them to lymphocytes in a form that they can recognize. APCs take up antigens (e.g., dendritic cells, macrophages, B cells, even tissue cells) in various ways. Some are collected in the periphery and transported to the secondary lymphoid tissues; other APCs normally reside in lymphoid tissues and intercept antigen as it arrives. B cells recognize antigen in a native form.

There are two major pathways of antigen processing for the APC and target cell, endogenous and exogenous. The **endogenous pathway** processes proteins that have been internalized, processed into fragments, and reexpressed at the cell surface membrane in association with MHC molecules. In this pathway, proteins in the cytoplasm are cleaved into peptide fragments about 20 amino acids in length. These fragments are then transported into the lumen of the endoplasmic reticulum by the transporter associated with the antigen-processing complex, where the fragments encounter newly formed, heavy-chain molecules of MHC class I and their associated beta2-microglobulin (β_2m) light chains. The heavy chain, light chain, and peptide form a trimeric complex, which is then transported to and expressed on the cell surface.

T cells that express the CD8+ cell surface marker recognize antigens presented by MHC class I molecules. CD8+ functions as a coreceptor in this process, binding to an invariant region of the MHC class I molecule. Pathogen clearance requires that CD8+ effector cells produce inflammatory cytokines and develop cytolytic activity against infected target cells, after which a small number of memory cells survive that rapidly regain effector function in the event of rechallenge. During this process, a relatively homogeneous pool of naïve CD8+ T cells differentiates into heterogeneous pools of effector and memory CD8+ T cells.

In the **exogenous pathway,** soluble proteins are taken up from the extracellular environment, generally by specialized or so-called professional APCs. The antigens are then processed in a series of intracellular acidic vesicles called endosomes. During this process, the endosomes intersect with vesicles that are transporting MHC class II molecules to the cell surface. CD4+ T cells recognize antigens that are presented by MHC class II molecules. As with CD8, the CD4 molecule functions as a coreceptor, increasing the strength of the interaction between the T cell and APC.

For both systems of antigen presentation, recognition of the antigen by the T cells is described as being MHC-restricted, a process whereby T cells recognize only antigen presented by self MHC molecules.

Antigen Recognition by T Cells

T cells are clonally restricted, so that each T cell expresses a receptor that can interact with a given peptide. Each lymphocyte makes only one type of antigen receptor and can recognize only a very limited number of antigens. Because receptors differ on each clone of cells, the entire lymphocyte population has an enormous number of different, specific antigen receptors.

The TCR of most T lymphocytes is composed of an alpha and beta polypeptide chain, with constant regions located close to the cell surface and the part that binds to the antigenic peptide of appropriate fit located away from the cell surface. The difference in structure of the distal regions of the alpha and beta chains allows the development of different clones of T cells. The TCR reacts with antigen in the context of MHC class I or II molecules on an APC (Fig. 4-13).

T cells recognize protein antigens in the form of peptide fragments presented at the cell surface by MHC I or II molecules. When the antigen-specific TCR on the T cell surface (specifically the zeta-beta chains) of the CD3 complex interacts with the appropriate peptide-MHC complex, it triggers phosphorylation of the intracellular domains of the CD3 zeta chains. Subsequently, the zeta-associated protein 70 (ZAP-70) binds to the phosphorylated zeta chains and is activated.

Simultaneous colligation of the cell marker CD4 (or CD8) with the MHC class II (or I) molecule results in the phosphorylation of particular kinases. These events stimulate the activation of at least three intracellular signaling cascades. T cell activation also requires a second costimulatory signal (e.g., interaction between marker CD28 on T cells and marker CD80 on APCs). This interaction also triggers several intracellular signaling pathways.

T Cell Activation

T cell activation requires a minimum of two signals:

- Signal 1 is delivered by the TCR-CD3 complex through interaction of the TCR α and β chains as they recognize peptide presented by a class I CD8+ T cell or a class II CD4+ T cell MHC molecule.
- Signal 2 is usually provided by the engagement of CD28 on the T cell with the costimulatory molecule CD80 or CD86 on the APC. The surface markers CD 137 and CD134 also provide costimulation to T cells.

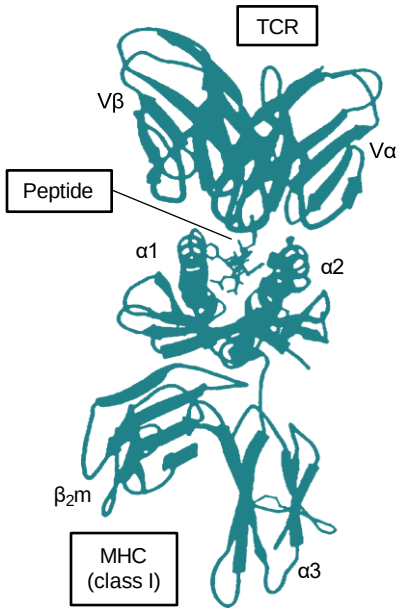


Figure 4-13 Recognition of peptide-MHC by TCR. This ribbon diagram is drawn from the crystal structure of the extracellular portion of a peptide-MHC bound to a T cell antigen receptor (TCR) that is specific for the peptide displayed by the MHC molecule. The peptide can be seen attached to the cleft at the top of the MHC molecule, and one residue of the peptide contacts the variable (V) region of a TCR. *(Adapted from Bjorkman PJ: MHC restriction in three dimensions: a view of T cell receptor/ligand interactions, Cell 89:167-170, 1997.)*

BOX 4-1 Screening for Congenital Immunodeficiencies

Components
CD2
CD3
HLA-DR
CD4
CD45RA
CD45RO
CD 8
CD4/CD8 ratio
CD19
NK cells

Adapted from Associated Regional and University Pathologists (ARUP): Lymphocyte subset panel 7: congenital immunodeficiencies, 2012 (http://www.aruplab.com/guides/ug/tests/0095899.jsp).

The optimal combination of effector function, proliferation, and survival requires both signals. Delivery of signal 1 without costimulation, which often occurs in tumor-infiltrating lymphocytes, leads to **anergy** and apoptosis, which limits the antitumor response of the cells.

Activation of T cells can lead to the following:

- Cell division
- Cytokine secretion by T cells
- Expression by T cells of antigens associated with activated state

Activated T cells frequently express activation antigens (Box 4-1). Expression of CD69 occurs within 12 hours of activation,

followed by CD25 (IL-2 receptor) and CD71 (transferrin receptor) in 1 to 3 days. Alternatively, in the case of Tc cells, interaction with antigen through the specific TCR leads to destruction of target cells.

If a cell does not receive a full set of signals, it will not divide and may even become anergic. Peripheral T cells generally exist in a resting state (G0 or G1). T cell activation is a complex reaction involving transmembrane signaling and intracellular enzyme activation steps. It is through soluble cytokines that T cell regulation influences the action of other T cells, accessory cells, and nonimmune constituents. When activated by the proper signals, T cells may carry out one or more of the following functions:

1. Proliferation
2. Differentiation
3. Production of cytokines
4. Development of effector function

T-Independent Antigen Triggering

Some antigens, particularly polysaccharide polymers (e.g., dextran), can trigger B cells without help from T cells. These **T-independent antigens** generally are not strong, provoke mainly IgM responses, and induce minimal immunologic memory.

NATURAL KILLER AND K-TYPE LYMPHOCYTES

A subpopulation of circulating lymphocytes (≈10%), NK and K-type lymphocytes, lack conventional antigen receptors of T or B cells. These cells are classified as effector lymphocytes that produce mediators (e.g., IL-2).

Although these cells were previously classified as null cells, MAbs demonstrate that NK and K-type cells express a variety of surface membrane markers (Table 4-3). Most of these cells lack CD3 but express CD2, CD16, CD56, CD57 and, occasionally, CD8.

Natural Killer Cells

Natural killer (NK) **cells** are essential mediators of virus immunity. Their deficiency in humans lead to uncontrolled viral replication and poor clinical outcome. MHC class I (MCH I) is essential to NK and T cell effector and surveillance functions. A total of 70% to 80% of NK cells have the appearance of large granular lymphocytes (LGLs). Up to about 75% of LGLs function as NK cells and LGLs appear to account fully for the NK activity in mixed cell populations.

Table 4-3 **Natural Killer Cell Profile**

Components	Reference Interval (% Positive)
CD2	75-92
CD3	63-84
CD5	61-88
CD7	73-94
CD8	14-39
CD16	1-12
CD56	7-27
CD57	1-26

Data from Associated Regional and University Pathologists (ARUP): Interpretive data guide, ed 2, Salt Lake City, Utah, 1999, Associated Regional and University Pathologists, p 473.

NK cells destroy target cells through an extracellular nonphagocytic mechanism referred to as a cytotoxic reaction, MHC-unrestricted cytolysis. Target cells include tumor cells, some cells of the embryo, cells of the normal bone marrow and thymus, and microbial agents. Studies have suggested that a considerable number of NK cells may be present in other tissues, particularly in the lungs and liver, where they may play important roles in inflammatory reactions and in host defense, including defense against certain viruses (e.g., cytomegalovirus, hepatitis). NK cells will actively kill virally infected target cells and, if this activity is completed before the virus has time to replicate, a viral infection may be stopped.

Several cytokines affect NK cell activation and proliferation. NK cells are highly responsive to IL-2, IL-7, and IL-12. These cytokines generate high cytokine-activated killer activity in these cells. In addition, NK cells synthesize a number of cytokines involved in the modulation of hematopoiesis and immune responses and in the regulation of their own activities.

Target cell recognition and the molecular identification and analysis of the involved NK cell receptors are undergoing intensive research. These molecules are mainly classified under the family of cell adhesion molecules (CAMs). The main class of effector CAMs shown to mediate NK cell functions is the leukocyte integrins—more specifically, the β_2 class of integrins.

Several NK cell surface molecules involved in target cell recognition and binding have been identified. NK cells recognize targets using several cell surface molecular receptors (e.g., CD2, CD69, NKR-P1) and a high density of the Fc receptor CD16 of IgG (FC-R III). They also receive inhibitory signals from MHC class I on potential target cells, transduced by a killer inhibitory receptor on the NK cell. CD56 may mediate interactions between effector and target cells. NK cells are able to bind and lyse antibody-coated nucleated cells through a membrane Fc receptor that can recognize part of the heavy chain of immunoglobulins. This enables NK cells to mediate antibody-dependent, cell-mediated cytotoxic (ADCC) activities. Some, if not all, of the activation of NK cells is mediated by CD16, which exerts a regulatory role in their cytolytic function. NK cells respond to cross-linking of CD16 and CD69 as follows:

- Increasing the rate of proliferation of NK cells
- Elevating the levels of TNF production within 4 hours of stimulation
- Increasing the expression of CD69 on the cell surface of NK cells
- Increasing the cytotoxicity activity against a normally resistant cell line (P815)

K-Type Lymphocytes

K-type killer cells are mononuclear cells that can kill target cells sensitized with antibody, which they engage through their

Fc receptors. Most K-type cells are non-T, non-B lymphocytes, but macrophages and eosinophils can also have K cell activity. K-type cells exhibit a different cytotoxic mechanism than NK cells. The target cell must be coated with low concentrations of IgG antibody, referred to as an ADCC reaction. An ADCC reaction may be exhibited by both K cells and phagocytic and nonphagocytic myelogenous-type leukocytes. K cells are capable of lysing tumor cells. Although morphologically similar to a small lymphocyte, the precise lineage of the K cell is uncertain.

DEVELOPMENT AND DIFFERENTIATION OF B LYMPHOCYTES

B cells represent a small proportion of the circulating peripheral blood lymphocytes. The unfavorable image of B lymphocytes in the pathogenesis of immune disease has been associated mainly with their capacity to produce harmful antibodies after differentiation into plasma cells.

Other roles have been discovered for B lymphocytes, including an antibody-independent pathogenic role of B cells (e.g., capability to present antigen). On recognition of a specific antigen, the B cell membrane is reorganized, resulting in an aggregation of B cell receptors in an immunologic synapse that functions as a platform for internalization of the complex. Internalized antigen is degraded and subsequently exposed to the B cell surface in association with MHC complex molecules for presentation to T cells. This surface presentation of antigen, in the presence of various costimulatory molecules, elicits the assistance of T cells required to assist B cell maturation, which in turns allows B cells to drive optimal T cell activation and differentiation into memory subsets.

B cells also have the capacity to expand clonally, which allows them to become the numerically dominant APCs. Activated B cells also produce a wide range of cytokines and chemokines that modulate the maturation, migration, and function of other immune effector cells.

B LYMPHOCYTE SUBSETS

B1 and B2 cells are B cell subsets. B1 cells are distinguished by the CD5 marker, appear to form a self-renewing set, respond to a number of common microbial antigens, and occasionally generate autoantibodies. B2 cells account for most of the B lymphocytes in adults. This subset generates a greater diversity of antigen receptors and responds effectively to T-dependent antigen.

B cells are derived from progenitor cells through an antigen-independent maturation process occurring in the bone marrow and GALT. Participation of B cells in the humoral immune response is accomplished by reacting to antigenic stimuli through division and differentiation into plasma cells. Plasma cells or antibody-forming cells are terminally differentiated B cells. These cells are entirely devoted to antibody production, a primary host defense against microorganisms.

The specific antibodies produced are able to bind to infected cells, free organisms bearing the antigen, and then inactivate those cells or organisms and destroy them. The condition of hyperacute rejection of transplanted organs is also mediated by B cells. In addition, antigenic stimulation prompts B cells to multiply.

Cell Surface Markers

B lymphocytes are best known to express CD19 but not CD3 surface membrane markers. During B-cell differentiation in the bone marrow, the surface molecule CD19 appears early and remains on the B cell unit until it differentiates into a plasma cell. Four proteins on the surface of mature B cells-CD19, CD21, CD81, and CD225—from the CD19 complex.

Primitive B cell precursors have δ chains in their cytoplasm and no Ig on their surface. More differentiated (but still immature) B cells have intact cytoplasmic IgM and surface IgM. Mature B cells lose their cytoplasmic IgM and add surface IgD to the surface IgM. These changes appear to occur in the absence of antigen and depend on cytokines.

In humans, there is evidence of four types of B **cell surface markers:**

1. Ig receptor is the best studied B cell surface marker. This receptor is actually an antibody molecule with antigenic specificity. According to the clonal selection theory, B cells exist in the body with Ig receptors specific for antigen before exposure to the antigenic substances. When specific antigen exposure does occur, the antigen will select the B cell having an Ig receptor with the best fit.

After binding and cooperative interaction with T cells, B cells undergo transformation into plasma cells. The secreted antibody, in turn, has the same specificity as the Ig receptor on the B cell. Almost all the antibody produced by plasma cells is secreted (plasma cells have few Ig receptors), but 90% of the antibody produced by B cells is expressed as surface Ig receptors. Some antigens (e.g., lipopolysaccharides from some gram-negative organisms) can bind to the Ig receptor and also stimulate an antibody response independent of T cell cooperation (T-independent antigens). This type of response is generally of low intensity and is class-restricted to the production of IgM antibody.

B cells have **surface immunoglobulin (sIg),** except for very immature lymphocytes and mature plasma cells, that are normally polyclonal (i.e., kappa and lambda light chains are present on the cytoplasmic membrane of B cells). Mu and delta heavy chains are usually found with kappa or lambda chains on any one cell surface. Gamma and alpha chains are rarely found on the surface of properly prepared, normal lymphocytes.

2. An Fc receptor that specifically binds the Fc portion of IgG antibody may function to aid B cells in binding to antigen already bound to antibody.
3. Receptors that bind fragments of the cleaved complement component C3 have been reported on the surface of approximately 75% of B cells. This receptor binds C3b, iC3b (inactivated C3b), and C3d, but the function of these receptors is not completely understood.
4. B cell surface antigens coded by the MHC class II genes are a fourth type of human B cell marker.

B Cell Activation

B cells can be stimulated in their resting state to enlarge, develop synthetic machinery, divide, mature, and secrete antibody. The proper signals for this sequence depend on the type of triggers, which can be specific or nonspecific and polyclonal. Specific activation involves the antigen that is complementary to the particular Ig on the surface. Nonspecific activation occurs with B cell mitogens.

Efficient antibody production to complex protein antigens requires T cell help, which in turn develops from APCs presenting antigen to the T cell. Activated T cells secrete a variety of cytokines that together with the specific antigen, trigger the B cell to develop into an antibody-secreting cell. This process also involves class switching.

In the immune response to a foreign protein, the first antibodies to appear are of the IgM class (or isotype). As the response proceeds, other isotypes (IgG, IgA, and IgE) emerge from Ig class switching. The isotype switch has considerable clinical importance because each of the four major isotypes has specialized biologic properties. IgG is the principal class of antibody in interstitial fluids and IgA is the protective antibody of mucosal surfaces. Isotype switching requires collaboration between antibody-synthesizing B cells and helper CD4+ T cells. The B cell uses IgM molecules on its surface to capture the antigen and present the antigen to the T cell. Contact between the collaborating lymphocytes is enhanced by complementary pairs of CAMs. Some CAMs (e.g., CD4, MHC class II antigens) are constitutively expressed on the surface of T and B cells, whereas others are induced. For example, contact between B and T cells induces the T cell to express a ligand for the B cell surface molecule CD40. In turn, CD40 interacts with the newly expressed CD40 ligand on the T cell, which leads to the expression of another B cell surface molecule, B7. The latter's partner on the surface of the T lymphocyte is CD28. These cooperative and synergistic interactions between T and B cells induce the secretion of cytokines such as IL-2 and IL-4.

Isotype switching requires two signals. The first is delivered by an interleukin and the second by the binding of CD40 to its ligand on the T cell. In the process of switching from IgM synthesis to IgE synthesis, IL-4 makes the IgE gene in the B cell accessible to the switch machinery initiated when CD40 binds to its ligand. In this process, the gene that encodes the variable region (the part of the antibody molecule that contains the antigen-binding site) moves from its position near the gene that encodes for IgM to a position near the gene that encodes for IgE.

PLASMA CELL BIOLOGY

The function of **plasma cells** (see Color Plate 9) is the synthesis and excretion of immunoglobulins. Plasma cells are not normally found in the circulating blood but are found in the bone marrow in concentrations that do not normally exceed 2%. Plasma cells arise as the end stage of B cell differentiation into a large, activated plasma cell.

The pathway from the B lymphocyte to the antibody-synthesizing plasma cell forms when the B cell is antigenically stimulated and undergoes transformation because of the stimulation of various **interleukins.** The immune antibody response begins when individual B lymphocytes encounter an antigen that binds to their specific Ig surface receptors. After receiving an appropriate second signal provided by interaction with helper T cells, these antigen-binding B cells undergo transformation and proliferation to generate a clone of mature plasma cells that secretes a specific type of antibody.

An increase in plasma cells can be seen in a variety of nonmalignant disorders, such as viral disease (e.g., rubella, infectious mononucleosis), allergic conditions, chronic infections, and collagen diseases. In plasma cell **dyscrasias,** the plasma cells can be greatly increased or infiltrate the bone marrow completely (e.g., multiple myeloma, Waldenström's macroglobulinemia).

Antibody molecules secreted by plasma cells consist of four chains—two light chains and two heavy chains, based on molecular weight—and can be enzymatically cleaved into Fab (antigen-binding) and Fc (crystallizable) fragments. The Fab portion binds antigen and contains the light chains and their antigenic markers (kappa, lambda), as well as heavy chains.

The Fc fragment contains the markers that distinguish the different classes of antibody and sites that will bind and activate complement and bind to Fc receptors on cells. The amino acid sequence for most of the antibody protein is constant, except for the antigen-binding portion of the molecule, which has a hypervariable region and accounts for the various antigenic specificities that the antibody is programmed to recognize.

ALTERATIONS IN LYMPHOCYTE SUBSETS

The normal functioning of helper cells and suppressor cells in the immune response can be reversed under certain conditions. For example, the target cell for human T cell leukemia or human immunodeficiency virus (HIV) is phenotypically a helper cell but functionally a suppressor cell. Functionally, the helper-inducer subset of cells signals B cells to generate antibodies, control production and switching of types of antibodies formed, and activate suppressor cells.The **suppressor-cytotoxic lymphocytes** control and inhibit antibody production by suppressing helper cells or by turning off B cell differentiation. The normal ratio of helper cells and suppressor cells (≈2:1) can be reversed under certain conditions.

Changes With Aging

Except for inconsistent values seen in extremely old adults, the total number of T cells in the peripheral blood is relatively stable throughout adult life. However, there is a change in the distribution of T cell subpopulations. A decrease in the number of suppressor cells and an increase in the helper cell population are demonstrated in older adults.

The effect of aging on the immune response is highly variable, but the ability to respond immunologically to disease is age-related. Faulty immunologic reactions (e.g., aberrant functioning of **immunoregulatory cells, effector T cells,** and antibody-producing B cells) may contribute to poor immunity in older adults. Functional deficits of T lymphocytes have been identified with aging, causing impairment of cell-mediated

immunity. In addition, skin testing reveals decreases in the intensity of delayed hypersensitivity in older adults. The proliferative response of T lymphocytes to mitogens or antigens such as *Mycobacterium tuberculosis* or varicella-zoster virus is impaired.

A decrease in Th cells is the primary cause of the impaired humoral response in older adults. Although the total number of B cells and total Ig concentration remain unchanged, the serum concentration of IgM is decreased and IgA and IgG concentrations are increased.

EVALUATION OF IMMUNODEFICIENCY SYNDROMES

Although more than 50 genetically determined **immunodeficiency syndromes** have been reported since 1952, defects in immunity were considered rare until acquired immunodeficiency syndrome (AIDS) emerged more than 30 years ago. This growing list of primary and secondary diseases now encompasses all major components of the immune system, including lymphocytes, phagocytic cells, and complement proteins.

Older children and adults with recurrent upper and lower respiratory tract infections and/or diarrhea, abscesses, sepsis, or meningitis should be evaluated for immunodeficiency. Before proceeding with laboratory testing, primary care providers need to rule out the following:

- Anatomic or physical causes (e.g., foreign bodies, indwelling catheters)
- Cancer
- Connective tissue disease
- Diabetes
- Renal disease

Laboratory testing can then proceed with a complete blood cell (CBC) count, including a platelet count and erythrocyte sedimentation rate (ESR). These are among the most cost-effective screening tests. If the ESR is normal, chronic bacterial infection is unlikely. If the absolute neutrophil count is normal, congenital and acquired neutropenias and severe chemotactic defects are eliminated. If the absolute lymphocyte count is normal, the patient is not likely to have a severe T cell defect. The absolute lymphocyte count is the number of lymphocytes in the total white blood cell (WBC) population (Box 4-2).

Laboratory tests to screen for more common immunodeficiencies include immunoglobulin testing, complement testing, cell-mediated immunity testing, and the neutrophil function test. Additional laboratory testing should include a general metabolic panel to assess overall general health, HIV types 1 and 2, protein electrophoresis, sweat chloride, and pneumococcal antibody IgG titers pre- and postvaccine in patients with only recurrent sinopulmonary infections. Follow-up testing based on any initially abnormal results is presented in Tables 4-4 and 4-5. If all initial test results are normal, IL-1 receptor-associated kinase-4 (IRAK-4) deficiency screening or a Toll-like receptor function assay should be performed. If abnormal results are subsequently found, the diagnosis is an innate immune deficiency.

Cell-Mediated Immune System

Deficiencies of cell-mediated immunity are often suspected in individuals with recurrent viral, fungal, parasitic, and protozoal infections. Patients with AIDS exhibit some of the most severe manifestations of cell-mediated immunity (see Chapter 25).

One avenue of testing involves delayed hypersensitivity skin testing to determine the integrity of the patient's cell-mediated immune response. More than 90% of normal adults will react to one of the following antigens within 48 hours after antigen exposure: *Candida albicans, Trichophyton,* tetanus toxoid, mumps, and streptokinase-streptodornase. Reactivity to histoplasmin or purified protein derivative (PPD) is positive in patients with active infection or previous exposure to histoplasmosis or tuberculosis, respectively; therefore these tests are not useful for the assessment of anergy.

The number of T lymphocytes, the primary effector cells in cell-mediated reactions, can be determined by several techniques. Previously, the gold standard was the E rosette technique (erythrocyte rosette formation), but the development of flow cytometry with MAbs has replaced this technique. Testing for the functionality of lymphocytes is just as important as a quantitative count of CD4+ and CD8+ cells.

Box 4-2 Determination of Absolute Lymphocyte Count

Absolute number of lymphocytes = total leukocyte count × percentage (%) of lymphocytes
Total leukocyte count = 25 × 10^9/L
Relative percentage (%) of lymphocytes = 76%
Absolute number = 19 × 10^9/L

Table 4-4 Next Steps in Laboratory Evaluation of Suspected Immunodeficiency

Abnormal Initial Laboratory Result	Follow-Up Testing
Protein electrophoresis	Immunofixation electrophoresis monoclonal protein detection; quantitation and characterization of IgA, IgG and IgM, and Bence Jones protein; depending on individual results, additional testing may be needed
Sweat chloride	Cystic fibrosis (CFTR)—32 mutations with reflex to sequencing
Positive for HIV-1, HIV-2	HIV-1 antibody confirmation by Western blot
Pneumococcal antibodies absent after vaccination	Specific antibody deficiency

Adapted from Associated Regional and University Pathologists (ARUP) Consult: Immunodeficiency evaluation for chronic infections in adults and older children testing algorithm, 2012 (http://www.arupconsult.com/Algorithms/ChronicInfections Adults.pdf).

The in vitro diagnostic test (IVD; see later, "Assessment of Cellular Immune Status") is the newest approach to testing the functionality of T lymphocytes. It is important to recognize that CD4 counts do not always reflect the actual status of the patient's immune system. ImmuKnow (Cylex, Columbia, Md) is the first U.S. Food and Drug Administration (FDA)–approved immune function test. It is widely considered to be the gold standard of immune function testing.

The QuantiFERON-CMI kit (Cellestis, Valencia, Calif) is an in vitro assay for measuring cell-mediated immune functionality. The procedure is a single-step enzyme-linked immunosorbent assay (ELISA) to determine T cell responses by measuring IFN-γ levels in plasma. This is a specific marker cytokine for a cell-mediated or inflammatory immune response (e.g., bacterial, parasitic, or viral).

Research-Based Tests

Various procedures are research-based, including a lymphocyte antigen and mitogen proliferation panel. This type of procedure measures cytokine production by mononuclear cells in response to mitogen stimulation by IL-1β types 6 and 8 and TNF-α. Another method includes flow cytometry and the enzyme-linked immunosorbent spot assay (ELISPOT). Flow cytometry can be used in conjunction with intracellular cytokine staining with ^{3}H-thymidine to detect the T cell response to specific antigenic stimulation by multianalyte fluorescence detection. In addition, peptide–MHC complex tetramer or pentamer staining is used to quantify the number of T cells with a particular antigenic epitope based on the expression of a specific T cell receptor. A multiplex cytokine analysis (e.g., multiplex bead-based Luminex assay [Life Technologies, Grand Island, NY]) is being used to detect multiple cytokines in serum, plasma, or tissue culture supernatants.

Humoral System

The humoral system can be screened for abnormalities by quantitating the concentrations of IgM, IgG, and IgA. An initial simple screening can be determined by the presence and titer of antibodies to type A and B red blood cell (RBC) antigens.

IMMUNOLOGIC DISORDERS

A breakdown in any part of the immune mechanism can lead to disease. Disorders with an immunologic origin can involve progenitor cells, phagocytosis (see Chapter 3), T cells, B cells, or complement (see Chapter 5).

Immunologic disorders can be divided into primary processes (dysfunction in the immune organ itself) and acquired, or secondary, processes (disease or therapy causing an immune defect). A third category, diseases mediated through immune mechanisms, can also be included. Because of its complexity and contemporary importance, AIDS is discussed separately in Chapter 25. Other immunoproliferative and autoimmune disorders are discussed in Chapters 27 to 30.

Table 4-5 Next Steps in Evaluation of Suspected Immunodeficiency Based on Physical Findings

Physical Manifestations	Follow-Up Laboratory Assays	Differential Diagnosis
Recurrent severe viral or fungal infections (e.g., candidiasis, herpes)	Lymphocyte subset for congenital immunodeficiencies, lymphocyte antigen and mitogen proliferation panel, proliferation panel with cytokine responses to mitogens; testing for 12 cytokines	T cell deficiency, HIV, CD4 deficiency, adenosine deaminase deficiency, nucleoside phosphorylase deficiency, chronic mucocutaneous candidiasis
Recurrent severe sepsis, *Neisseria* spp., *Streptococcus pneumoniae* infections	Rule out previous splenectomy and immunoglobulin abnormality; then order a total CAEI. If abnormal (low) activity, analyze C2-C5 components.	Complement deficiency
Abscesses, pneumonia, recurrent respiratory infections with or without diarrhea	Order quantitative IgM, IgG and IgA, IgE, CAEI, neutrophil oxidative burst assay (DHR), leukocyte adhesion deficiency panel, and myeloperoxidase stain.	Low IgM- or IgG-hypogammaglobulinemia Low complement—complement defect Abnormal DHR—chronic granulomatous disease Decreased CD11b/CD18—LAD-1, Decreased CD15—LAD-2 Increased IgE—Possible hyper-IgE syndrome (Job syndrome) but must be followed by *Candida*-specific IgE neutrophil chemotaxis and subsequent genetic testing Positive neutrophil antibody—autoimmune neutropenia, Absence of myeloperoxidase—myeloperoxidase deficiency

Adapted from Associated Regional and University Pathologists (ARUP) Consult: Immunodeficiency evaluation for chronic infections in adults and older children testing algorithm, 2012 (http://www. arupconsult.com/Algorithms/ChronicInfections Adults.pdf).
CAEI, Complement activity enzyme immunoassay; *DHR*, neutrophil oxidative burst assay; *LAD-1*, leukocyte adhesion deficiency, type 1; *LAD-2*, leukocyte adhesion deficiency, type 2.

Immunodeficiency disorders may be caused by defects in the quality (defects) or quantity (deficiencies) of lymphocytes and may be congenital or acquired. These conditions may be combined disorders or may involve T cells or B cells (Table 4-6).

Primary Immunodeficiency Disorders

Primary immunodeficiencies (PID) are rare genetic disorders of the innate and adaptive immune system. Classic **primary immunodeficiency disorders (PIDs)** are usually monogenic (mendelian) disorders affecting host defenses (Box 4-3). More than 200 clinical phenotypes of PID have been described. Over 120 different gene mutations have been identified which cause impairment in the differentiation and/or function of immune cells with different degrees of severity. Diseases associated with a primary defect in the immune response are comprised of about 40% T cell disorders, 50% B cell disorders, 6% phagocytic abnormalities, and 4% complement alterations (Fig. 4-14). The most common T cell deficiency states are those associated with a concurrent B cell abnormality. Primary immunodeficiency disorders are predominantly seen (75%) in children younger than 5 years.

Gene therapy with hematopoietic stem cells (HSC) is a therapeutic strategy for the treatment of several forms of primary immunodeficiency. Current approaches use gene transfer of the therapeutic gene into autologus HSC by retroviral vector-mediated gene transfer. This method has been successful in severe combined immunodeficiencies (SCID-1) and chronic granulomatous disease (CGD). Wiskott-Aldrich syndrome is another good candidate for gene therapy treatment.

T Cell and Combined Immunodeficiency Disorders

DiGeorge's Syndrome

Cause. This T cell defect is a congenital anomaly that represents faulty embryogenesis of the endodermal derivation of the third and fourth pharyngeal pouches, which results in aplasia of the parathyroid and thymus glands. At autopsy, parathyroid and vestigial thymus glands may be found in ectopic locations. The newborn may exhibit various facial and vascular anomalies, collectively referred to as pharyngeal pouch syndrome. In addition to the established embryonic cause of DiGeorge's syndrome, a nutrient (zinc) deficiency in utero has been suggested as a cause of this process.

Signs and Symptoms. DiGeorge's syndrome is present at birth. Initial manifestations can include hypocalcemic tetany, unusual facies, and congenital heart defects. An increased susceptibility to viral, fungal, and disseminated bacterial infections (e.g., acid-fast

Box 4-3 Primary Immunodeficiency Diseases

T Cells
Combined immunodeficiency
Thymic alymphoplasia
Swiss type
Adenosine deaminase deficiency
Nezelof syndrome
DiGeorge's syndrome (thymic hypoplasia)
Wiskott-Aldrich syndrome
Chronic mucocutaneous candidiasis
Immunodeficiency associated with nucleoside phosphorylase deficiency
Short-limbed dwarfism
Ataxia-telangiectasia
Thymoma
Leukocyte adhesion deficiency (LAD)

B Cells
Selected IgA deficiency associated with:
Normal state
Allergy
Autoimmune disease
Central nervous system disease
Gastrointestinal disorders
Malignancy
Pulmonary infections
X-linked infantile agammaglobulinemia
X-linked immunodeficiency with hyper-IgM
Common variable hypogammaglobulinemia
Selective IgM deficiency
IgG subclass deficiency

Adapted from Graziano FM, Bell CL: The normal immune response and what can go wrong. A classification of immunologic disorders, Med Clin North Am 69:439–452, 1985.

Table 4-6 T Cell and B Cell Disorders

T Cell Disorder	B Cell Disorder
Congenital	
Thymic hypoplasia (DiGeorge's syndrome)	Bruton's agammaglobulinemia
Acquired	
Acquired immunodeficiency syndrome	Autoimmune disorders
Hodgkin's disease	Multiple myeloma
Chronic lymphocytic leukemia	
Systemic lupus erythematosus	

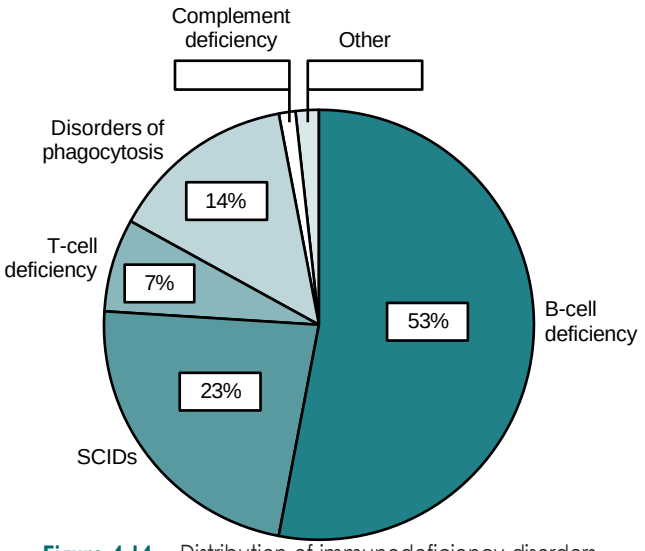


Figure 4-14 Distribution of immunodeficiency disorders.

bacilli, *Listeria monocytogenes, Pneumocystis jiroveci* [formerly known as *P. carinii*]) result from the defect of T cells normally controlled by cell-mediated immunity. Infants usually die of sepsis during the first year of life.

Immunologic Manifestations. Peripheral lymphoid tissue appears to be normal except for the depletion of T cells in thymus-dependent zones, such as subcortical region of the lymph nodes and perifollicular and periarteriolar lymphoid sheaths of the spleen. Lymph node paracortical areas and thymus-dependent regions of the spleen show variable degrees of depletion.

In the circulating blood, lymphopenia is generally present, although in some cases the concentration of lymphocytes is normal. However, an abnormally high CD4+/CD8+ ratio is present because of a decrease in CD8+ cells. Most patients with DiGeorge's syndrome have a decreased percentage of cells expressing the CD3+ (mature T cell) antigen. Because patients do demonstrate lymphocytes capable of differentiating to the more mature surface markers, such as CD4+, a small rudimentary thymus is believed to be present in these patients. Lymphocytic responsiveness to antigenic and mitogenic stimulation can be absent, reduced, or normal, depending on the degree of thymic deficiency. Cell-mediated immune reactions such as delayed hypersensitivity and skin allograft rejections, however, are absent or feeble.

Serum Ig concentrations are near normal. Levels of IgA may be diminished and of IgE may be elevated. Antibody response to primary antigenic stimulation may be unimpaired.

Nezelof Syndrome (Cellular Immunodeficiency With Immunoglobulins)

Cause. An autosomal recessive pattern of inheritance is often seen. The defect appears to exist on chromosome 14q13.1.

Signs and Symptoms. Nezelof syndrome is the PID most likely to be confused with AIDS in the pediatric age group. Infants have failure to thrive, recurrent or chronic pulmonary infections, oral or cutaneous candidiasis, chronic diarrhea, recurrent skin infections, gram-negative sepsis, urinary tract infections, and severe varicella.

Immunologic Manifestations. Nezelof syndrome is characterized by lymphopenia, neutropenia, and eosinophilia. In addition, diminished lymphoid tissue and abnormal thymus architecture are observed. Peripheral lymphoid tissues are hypoplastic and demonstrate paracortical lymphocyte depletion. Lymphocyte responses to mitogens, antigens, and allogeneic cells are profoundly depressed but not totally absent. Serum levels of most of the five Ig classes are normal or increased. Antibody-forming capacity has been reported as normal in one third of cases.

Severe Combined Immunodeficiency

Cause. Severe combined immunodeficiency (SCID) is caused by the inappropriate development of progenitor cells into lymphocyte precursors. This hereditary and invariably fatal disorder in infants results from the lack of both T and B cells and the consequent inability to synthesize antibody.

Mutations in the IL-2 receptor complex, a hematopoietic growth factor, have been shown to cause X-linked SCID in humans. Two modes of inheritance are known, autosomal recessive and X-linked recessive. X-linked recessive SCID is thought to be the most common form of SCID in the United States, which accounts for the 3:1 male-to-female ratio with the disorder.

Of patients with autosomal SCID, 50% have a concomitant deficiency of adenosine deaminase (ADA), an aminohydrolase that converts adenosine to inosine. Analysis by complementary (copy) DNA (cDNA) probe has revealed that the deficiency results from a hereditable point mutation in the ADA gene. Another variant with a severe deficiency in T cell immunity but normal B cell concentrations is associated with purine nucleotide phosphorylase deficiency.

There are two main forms of defective expression of MHC antigens. In a less common form of SCID, known as bare lymphocyte syndrome, an MHC class I antigen deficiency is present. In another form of defective expression, MHC class I antigen deficiency plus the absence of class II antigens is present. Patient lymphocytes cannot be typed by standard serologic cytotoxicity tests.

Signs and Symptoms. No important differences in signs and symptoms exist between the two major genetic types of SCID. Initial manifestations of SCID are repeated debilitating infections beginning within the first 6 months of life. These are dominated by bacterial, viral, and fungal infections of the respiratory and intestinal systems and skin. Infants with SCID usually die within 3 years of birth from lung abscesses, *Pneumocystis* pneumonitis, or a common viral disorder such as chickenpox or measles.

Immunologic Manifestations. The thymus and other lymphoid organs are severely hypoplastic. The bone marrow is devoid of lymphoblasts, lymphocytes, and plasma cells. Lymphocytes are also absent from lymphoid tissues such as the spleen, tonsils, appendix, and intestinal tract. Variable hypogammaglobulinemia with decreased serum IgM and IgA levels and poor to absent antibody production are representative features. Moderate lymphocytopenia is detectable early in infancy. T cell functions are decreased. The circulating blood contains no CD4+, CD8+, or CD3+ cells. The percentage of B cells is usually normal.

Patients with the X-linked form of SCID usually appear similar to those with the autosomal recessive form, except that they tend to have an increased percentage of B cells. However, the defect affects B-lineage cells as well as T-lineage cells.

Chronic Mucocutaneous Candidiasis

Cause. Chronic mucocutaneous candidiasis (CMC) results from a primary defect in cell-mediated immunity. T cells specifically fail to recognize only the *Candida* antigen.

Signs and Symptoms. Patients with CMC usually survive to adulthood. The characteristic manifestation is *Candida* infection of the mucous membranes, scalp, skin, and nails. Endocrine abnormalities, often polyendocrinopathies, are frequently associated with fungal manifestations. Sudden death from adrenal insufficiency has been reported in patients with CMC.

Immunologic Manifestations. Patients demonstrate normal skin reactions to testing with all antigens except *Candida.*

B Cell and Antibody Deficiency Disorders

Because the primary function of B cells is to produce antibody, the major clinical manifestation of a B cell deficiency is an increased susceptibility to severe bacterial infections. Selective

IgA deficiency is the most common B cell disorder, affecting 1 in 400 to 800 persons. Because IgA is the primary immunoglobulin in secretions, a deficiency contributes to pulmonary infections, gastrointestinal (GI) disorders, and allergic respiratory disorders. Most cases (50% of reported cases are associated with Ig deficiencies) are autoimmune in nature, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), thyroiditis, and pernicious anemia.

Bruton's X-Linked Agammaglobulinemia

Cause. This is a classic example of an X-linked agammaglobulinemia, in which a disease-causing variant in the gene coding for Bruton's tyrosine kinase (BTK) leads to the arrest of B cell development at the pre–B cell stage.

Signs and Symptoms. X-linked agammaglobulinemia occurs primarily in young boys, but scattered cases have been identified in girls. Manifestations begin in the first or second year of life. Hypersusceptibility to infection does not develop until 9 to 12 months after birth because of passive protection by residual maternal immunoglobulin. Thereafter, patients repeatedly acquire infections with high-grade extracellular pyogenic organisms such as streptococci. This disorder is characterized by sinopulmonary and central nervous system (CNS) infectious episodes and severe septicemia, but patients are not abnormally susceptible to common viral infections (excluding fulminant hepatitis), enterococci, or most gram-negative organisms. Chronic fungal infections are not usually present.

An autoimmune phenomenon, especially a juvenile RA type of disease, has also been associated with X-linked agammaglobulinemia. In addition, patients are highly vulnerable to a malignant form of dermatomyositis that eventually involves destructive T cell infiltration surrounding the small vessels of the CNS. In addition to infections and connective tissue disorders, agammaglobulinemic patients also have hemolytic anemia, drug eruptions, atopic eczema, allergic rhinitis, and asthma.

Immunologic Manifestations. The diagnosis of X-linked agammaglobulinemia is suspected if serum concentrations of IgG, IgA, and IgM are notably below the appropriate level for the patient's age. Tests for natural antibodies to blood group substances and for antibodies to antigens given during standard courses of immunization (e.g., diphtheria) are useful in distinguishing this disorder from transient hypogammaglobulinemia of infancy (see later).

B cells are almost absent from bone marrow and lymphoid tissues. A deficiency or absence of peripheral B lymphocytes is usually noted. If present, B cells are unresponsive to T cells and incapable of antibody synthesis or secretion. Surface immunoglobulins are absent. However, patients have normal numbers of CD3+ and CD8+ cells and many have normal CD4+ cells. Male children possess normal T cell function; therefore, homograft rejection mechanisms are intact and delayed-hypersensitivity reaction for both tuberculin and skin contact types can be elicited.

Common Variable Immunodeficiency

Cause. Common variable immunodeficiency (CVID) is a form of primary acquired agammaglobulinemia, occurring equally in males and females. The cause of CVID seems to be heterogeneous, with abnormalities of B cell maturation, antibody production, antibody secretion, or T cell regulation. Family clusters have been reported in which first-degree relatives of patients with selective IgA deficiency have a high incidence of abnormal Ig concentration, autoantibodies, autoimmune disease, and malignant neoplasms. Findings of rare alleles or deletions of MHC class III genes in patients with IgA deficiency of CVID have suggested that the susceptibility gene(s) is (are) on chromosome 6.

Signs and Symptoms. CVID usually manifests in the second or third decade of life. Signs and symptoms include frequent sinopulmonary infections, diarrhea, endocrine and autoimmune disorders, and malabsorption (e.g., of vitamin B_{12}). Intestinal giardiasis is also prevalent.

Immunologic Manifestations. Both the decreased concentration of immunoglobulins and near absence of serum and secretory IgA are thought to represent the most common and well-defined type of PID. The pattern of inheritance suggests that an autosomal function of antibodies is usually compromised. The number of B cells is typically normal or mildly depressed. Despite a normal number of circulating Ig-bearing B lymphocytes and the presence of lymphoid cortical follicles, blood lymphocytes do not differentiate into Ig-producing cells. In most patients the defect appears to be intrinsic to the B cell. The primary defect in Ig synthesis may be caused by the absence or dysfunction of CD4+ cells or by increased CD8+ supressor cell activity. Therefore, cellular immunity and Ig production are impaired by the interaction between helper and suppressor T cell subsets. Lymph nodes lack plasma cells, but may show striking follicular hyperplasia.

The total IgG level may be normal, but a subclass (usually IgG2 or IgG3) is deficient. Both IgA and IgM may be detectable, but IgM levels may be elevated. In addition, some patients may have thymoma and refractory anemia.

Immunoglobulin Subclass Deficiencies

Some patients have deficiencies of one or more subclasses of IgG despite a normal total IgG serum concentration. Most of those with absent or very low concentrations of IgG2 have been patients with selective IgA deficiency.

Selective Immunoglobulin A Deficiency

Cause. An isolated absence mode is often seen in pedigrees of individuals with CVID. IgA deficiency has been noted to evolve into CVID, and rare alleles and deletions of MHC class III genes in both conditions suggest a common basis.

Signs and Symptoms. IgA deficiency is typically associated with poor health. Infections occur predominantly in the respiratory, GI, and urogenital tracts. There is no clear evidence that patients have any increased susceptibility to viral agents. IgA deficiency has been noted in patients treated with phenytoin, sulfasalazine, penicillamine, and gold, suggesting that environmental factors may lead to expression of the defect.

Immunologic Manifestations. As many as 44% of patients with selective IgA deficiency demonstrate antibodies to IgA. Severe or fatal anaphylactic reactions after IV administration of blood products containing IgA and anti-IgA antibodies (particularly IgE anti-IgA antibodies) have occurred.

Immunodeficiency With Elevated Immunoglobulin M (Hyper-IgM)

Cause. A sex-linked mode of inheritance has been noted in some pedigrees. The abnormal gene in the X-linked type has been localized to Xq24-Xq27. However, more than one genetic cause is suspected.

Signs and Symptoms. Patients with hyper-IgM defect become symptomatic during the first or second year of life, with recurrent pyogenic infections, including otitis media, sinusitis, pneumonia, and tonsillitis. Hemolytic anemia and thrombocytopenia have been observed. Transient, persistent, or cyclic neutropenia is a common feature.

Immunologic Manifestations. This disorder is characterized by extremely low concentrations of IgG and IgA and, most frequently, greatly elevated concentrations of polyclonal IgM. Normal or slightly reduced numbers of IgM and IgD B lymphocytes have been observed.

Transient Hypogammaglobulinemia of Infancy Unlike patients with Bruton's X-linked agammaglobulinemia or CVID, patients with transient hypogammaglobulinemia of infancy can synthesize antibodies to A and B erythrocyte antigens, if they lack the antigen(s), and to diphtheria and tetanus toxoids. Antibody production usually occurs by 6 to 11 months of age. This antibody production occurs before Ig levels become normal.

X-Linked Lymphoproliferative Disease (Duncan's Disease)

Cause. Duncan's disease is caused by a recessive trait. The defective gene has been localized to the Xq26-Xq27 region.

Signs and Symptoms. The disease is characterized by an inadequate immune reaction to infection with Epstein-Barr virus (EBV). Infected patients are apparently healthy until they experience infectious mononucleosis (see Chapter 22). Two thirds of more than 100 patients studied died of overwhelming EBV-induced B cell proliferation during mononucleosis. Most patients surviving the primary infection developed hypogammaglobulinemia and/or B cell lymphomas.

Immunologic Manifestations. A marked impairment in the production of antibodies to the EBV nucleus has been noted in affected patients. In contrast, titers of antibodies to the viral capsid antigen range from zero to extremely elevated.

Antibody-dependent, cell-mediated cytotoxicity against EBV-infected cells has been low in many patients. NK cell function is also depressed. There is also a deficiency in long-lived T cell immunity to EBV.

Partial Combined Immunodeficiency Disorders

Wiskott-Aldrich Syndrome (Immunodeficiency With Thrombocytopenia and Eczema)

Cause. The primary defect in this uncommon X-linked recessive pediatric disease is caused by a mutation of the gene encoding Wiskott-Aldrich syndrome protein (WASp), which plays a critical role in actin polymerization in blood cells. The mutated gene is expressed uniquely in hematopoietic cells. The effects of WAS gene mutation on this process are of interest particularly, because the actin cytoskeleton has a prominent role in the basic mechanisms of cell adhesion and migration.

Signs and Symptoms. Wiskott-Aldrich syndrome is characterized by the triad of thrombocytopenic purpura, increased susceptibility to infection, and eczema (atopic dermatitis). Affected boys rarely survive beyond 10 years of age. Thrombocytopenia and bleeding are common. Platelets are small, with an intrinsic defect. Patients usually die from sepsis, hemorrhage, or malignancy.

Immunologic Manifestations. There is a tendency toward the development of autoimmune disease. Progressive deterioration of the thymus leads to a defect in cellular immunity and the attrition of T cell populations from the lymph nodes and spleen. Decreased numbers of T cells and alteration in the normal T4:T8 ratio of lymphocytes are manifested. Serum levels of IgM are low, but IgG concentrations are usually normal. IgA levels are normal or elevated, and IgE levels are usually elevated.

The lymph nodes and spleen of WAS patients show relative depletion of lymphocytes from T cell areas. Depletion of the splenic and circulating marginal zone B cells is characteristic and may explain the defective antibody responses, particularly to polysaccharide antigens.

Hereditary Ataxia-Telangiectasia

Cause. This autosomal recessive disorder apparently results from the coexistence of a T cell deficiency with a defect in DNA repair, which leads to extreme, nonrandom chromosomal instability. The sites of chromosomal breakage involve chromosomes 7 and 14 in more than 50% of patients.

Signs and Symptoms. Ataxia-telangiectasia is characterized by ataxia and choreoathetosis in infancy. Multiple telangiectases appear on exposed oculocutaneous surfaces during childhood. A high incidence of malignancy (e.g., lymphoma) is also seen. Children with this disorder eventually die of respiratory insufficiency and sepsis.

Immunologic Manifestations. The thymus is hypoplastic or **dysplastic,** and the thymus-dependent zones of the lymph nodes are void of cells. About 80% of patients lack serum and secretory IgA and some develop IgG antibodies to injections of IgA. The signs and symptoms of the disease appear to result from a concomitant T cell deficiency, deficiency of DNA repair, and disordered IgG synthesis.

Hyperimmunoglobulinemia E Syndrome

Cause. This disorder has a presumed autosomal dominant pattern of inheritance.

Signs and Symptoms. Hyperimmunoglobulinemia E syndrome is a relatively rare PID characterized by recurrent severe staphylococcal abscesses. From infancy, patients have histories of staphylococcal abscesses involving the skin, lungs, joints, and other sites; persistent pneumatoceles develop as a result of the recurrent pneumonias. Pruritic dermatitis also occurs.

Immunologic Manifestations. Patients have elevated levels of serum IgE and IgD; usually, normal concentrations of IgG, IgA, and IgM are present. Poor antibody and cell-mediated responses to **neoantigens** are demonstrated. In addition, a decreased percentage of T cells with the memory receptor CD45RO has been noted.

T Cell Activation Defects

Some patients with defective activation of T cells have experienced the following:

- Defective surface expression of the CD3-TCR complex caused by mutation in the gene encoding the CD3 γ subunit
- Defective signal transduction from the TCR to intracellular metabolic pathways
- Pretranslational defect in IL-2 or other cytokine production

These conditions are characterized by the presence of T cells that appear phenotypically normal but fail to proliferate or produce cytokines in response to stimulation with mitogens, antigens, or other signals delivered to the T cell antigen receptor. Patients' symptoms are similar to those of other T cell–deficient individuals; some patients with severe T cell activation defects may clinically resemble patients with SCID.

Other Primary Immunodeficiencies

In addition to hereditary or congenital disorders of lymphocytes, several PIDs involve the complement system and phagocytic cells. **Complement Deficiency.** Deficiencies in all of the components of the complement system have been described (see Chapter 5). These deficiencies are genetic in origin. Unusual susceptibility to infection is characteristic of some of these components, particularly deficiencies involving C3, C5, C6, and C7 (Table 4-7).

A functional deficiency of polymorphonuclear neutrophil (PMN) leukocytes is chronic granulomatous disease (CGD; see Chapter 3). This fatal syndrome usually begins with the onset of symptoms during the first year of life.

Secondary Immunodeficiency Disorders

A secondary immunodeficiency can result from a disease process that causes a defect in normal immune function, which leads to a temporary or permanent impairment of one or multiple components of immunity in the host (Box 4-4). Patients with secondary immunodeficiencies, which are much more common than primary deficiencies, have an increased susceptibility to infections, as seen in the PIDs.

Immunosuppressive agents and burns are major causes of secondary immunodeficiencies. In varying degrees, immunosuppressive agents have been demonstrated to affect every component of the immune response. In burn patients, septicemia is a common complication in those who survive the initial period of hemodynamic shock. The mechanism that seems most critical in thermal injury is disruption of the skin; however, interference with phagocytosis and deficiencies of serum Ig and complement levels have also been observed.

Immune-Mediated Disease

The immune system is normally efficient in eliminating foreign antigens. The nature of the antigen or the genetic makeup of the host, however, can cause alterations of the immune response that can be injurious and lead to immune-mediated disease (Table 4-8). In these disorders, the immune response is normal but the reactivity is heightened, prolonged, or inappropriate.

A major concern is allergic reactions, characterized by an immediate response on exposure to an offending antigen and the release of mediators (e.g., histamine, leukotrienes, prostaglandins) capable of initiating signs and symptoms (see Chapter 26). Although allergic reactions are associated with IgE, not all allergic reactions are IgE-mediated. Complement activation by immune complexes or through the alternative complement pathway has been shown to release complement C3a and C5a anaphylatoxins, which are capable of producing similar reactions.

Table 4-7 Complement Deficiencies

Deficient Component	Common Types of Infections
C1 (r/q)	Gram-positive, mainly respiratory
C2	Gram-positive, recurrent respiratory; meningitis, sepsis, tuberculosis
C3	Gram-positive, recurrent
C4	Gram-positive; sepsis, meningitis
C5	Meningitis *(Neisseria meningitidis)*, disseminated gonococcal infection
C6	Meningitis *(N. meningitidis)*, disseminated gonococcal infection
C7	Meningitis *(N. meningitidis)*
C8	Meningitis *(N. meningitidis)*, disseminated gonococcal infection
C9	Meningitis *(N. meningitidis)*

Box 4-4 Secondary Immunodeficiencies

Hematologic Lymphoproliferative Disorders
- Hodgkin's disease and lymphoma
- Leukemia, myeloma, macroglobulinemia
- Agranulocytosis and aplastic anemia
- Sickle cell disease

Other Systemic Processes and Metabolic Disorders
- Nephrotic syndrome
- Protein-losing enteropathy
- Diabetes mellitus
- Malnutrition
- Hepatic disease
- Uremia
- Aging

Viral Infection
- AIDS

Surgical Procedures and Trauma
- Splenectomy
- Burns

Immunosuppressive Agents
- Antimetabolites
- Corticosteroids
- Radiation

Adapted from Graziano FM, Bell CL: The normal immune response and what can go wrong. A classification of immunologic disorders, Med Clin North Am 69:439–452, 1985.

Table 4-8 Immune-Mediated Disease

Type	Cause, Disease
Allergic hypersensitivity	Foods, drugs, aeroallergens (dust, pollens, molds)
Contact hypersensitivity	Poison ivy, nickel, cosmetics
Transfusion Reactions	
Autoimmune disease	Systemic lupus erythematosus, rheumatoid arthritis, vasculitis syndromes, hemolytic anemia, idiopathic thrombocytopenia, pernicious anemia, Goodpasture's syndrome, myasthenia gravis, Graves' disease

Autoimmune disease is thought to be caused by antibody or T cell sensitization with autologous self-antigens (see Chapter 28). Postulated mechanisms of this process include the following:

- Altered antigen or neoantigen. These antigens may be created by chemical, physical, or biologic processes. Hemolytic anemia caused by a drug interaction is an example of this process occurring in RBCs.
- Shared or cross-reactive antigens. Evidence has suggested that poststreptococcal disease occurs through this mechanism.

Autoimmune Lymphoproliferative Syndrome

Autoimmune lymphoproliferative syndrome (ALPS) is a disease in which a genetic defect in programmed cell death, or apoptosis, leads to breakdown of lymphocyte homeostasis and normal immunologic tolerance. ALPS is the first pediatric syndrome described in which the primary defect is in apoptosis.

Defective apoptosis in lymphocytes (and, in ALPS type II, dendritic cells) leads to accumulation of these cells in the lymphoid organs after they would normally be eliminated. As a result, cells with autoimmune potential are unchecked and can induce a variety of autoimmune diseases; the risk for malignant transformation to lymphoma is increased.

Patients with ALPS have chronic enlargement of the spleen and lymph nodes, various manifestations of autoimmunity, and elevation of a normally rare population of double-negative T cells (DNTs). When lymphocytes from ALPS patients are cultured in vitro, they are resistant to apoptosis, as compared with cells from healthy controls.

Most ALPS patients have mutations in a TNF receptor gene that is a member of a superfamily (TNFRSF6). This gene, previously known as *APT1*, encodes the cell surface receptor for the major apoptosis pathway in mature lymphocytes.This receptor has many names, including Fas. The Fas apoptotic pathway is important for eliminating excess T cells after they have been activated and also eliminating antigen-driven and autoreactive T cell clones. Fas is a functional trimer residing at the cell membrane that when engaged by trimeric Fas ligand (FasL), initiates a proteolytic cascade leading to chromosomal DNA degradation and cell death.

CASE STUDY

History and Physical Examination

A 33-year-old man, the child of unrelated parents of Mexican descent, was examined because of a history of frequent sore throats and sinus headaches. Recently, he had a severe bout of pneumonia and was just diagnosed with bacterial conjunctivitis and chronic gastritis caused by *Helicobacter pylori*.

Laboratory Data

Assay	Patient's Result	Reference Range
Total leukocytes	7.2×10^9/L	$4.5\text{-}9.0 \times 10^9$/L
Total lymphocytes	3.2×10^9/L	$2.7\text{-}5.4 \times 10^9$/L
T lymphocytes	3.15×10^9/L	$2.7\text{-}5.3 \times 10^9$/L
B lymphocytes	Too low to count, almost undetectable	
Serum Immunoglobulins		
IgM	0.03g/L	3.0-15.8g/L
IgG	0.31g/L	0.4-2.2g/L
IgA	Not detectable	0.15-1.3g/L
IgE	Not detectable	<100IU/mL

Assay	Patient
Blood group	O
Anti-A and anti-B titer	1:2, very low
Serum immunoglobulin (mg/dL)	
IgM	45, low
IgG	200, very low
IgA	23, very low

Questions

1. A laboratory result of significance to the diagnosis of this patient is:
   a. Low or absent immunoglobulin levels
   b. Extremely low B-lymphocyte count
   c. Low T-lymphocyte count
   d. Both a and b
2. When vaccinated, this boy's immune system should:
   a. Recognize the vaccine as a foreign antigen
   b. Mount a weak antibody response to a vaccine
   c. Fail to recognize the vaccine as a foreign antigen
   d. Both a and b

See Appendix A for the answers to these questions.

Continued

CASE STUDY—Cont'd

Critical Thinking Group Discussion Questions

1. What abnormalities are evident in the laboratory assay results?
2. Does the patient's history suggest a genetic abnormality?
3. What kind of response would be expected from a vaccination?

See Instructor Evolve for discussion of the answers to these questions.

Assessment of Cellular Immune Status*

Transplantation Immune Cell Function Assay ImmuKnow, Cylex Inc., Columbia, MD

Principle

Phytohemagglutinin (PHA) is a nonspecific mitogen that can be used to stimulate cell division in CD4 T lymphocytes, regardless of their antigenic specificity or memory status. Therefore, PHA is considered to be a global stimulator of the immune system. The production of intracellular adenosine triphosphate (ATP) is one of the first steps in cellular activation following stimulation with mitogens such as PHA. ATP is a multifunctional nucleotide that plays an indispensable role in the transfer of intracellular chemical energy.

When a sample of patient blood is incubated with PHA, increased ATP production (see figure below) occurs within PHA-activated CD4 T cells. These cells are then isolated by the addition of magnetic beads coated with anti-CD4 monoclonal antibody. The isolated CD4 cells are washed on a magnetic tray and lysed to release intracellular ATP. The amount of measured light emitted following the addition of a luminescence reagent is proportional to the amount of ATP present. An established calibration curve is used to characterize the cellular immune function of the sample.

Procedural Protocol

*See Evolve for a complete description of the method.

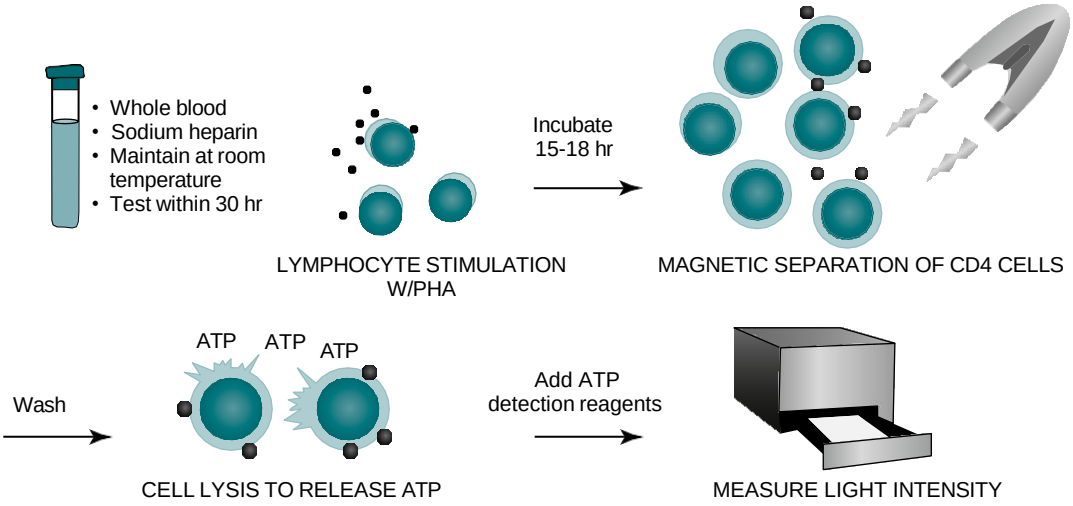
Results

ATP Level Results	Risk of Infection	Risk of Rejection
Low	Increased	Decreased
Moderate	Normal	Decreased
Strong	Normal	Increased

Adapted from Associated Regional and University Pathologists (ARUP) Laboratories, 2012 (http://www.aruplab.com).

CHAPTER HIGHLIGHTS

- Lymphocytes represent the cellular components of the specific system of body defense. These cells function cooperatively in cell-mediated or humoral immunity.
- The primary lymphoid organs in mammals are the bone marrow (and/or fetal liver) and thymus.
- The secondary lymphoid tissues include the lymph nodes, spleen, and Peyer's patches in the intestine. Proliferation of the T and B lymphocytes in the secondary or peripheral lymphoid tissues is primarily dependent on antigenic stimulation.
- Several major categories of lymphocytes are recognized by the presence of cell surface membrane markers. These categories are T and B cells and natural killer (NK) and K-type lymphocytes.
- The function of plasma cells is the synthesis and excretion of immunoglobulins.
- Monoclonal antibody (MAb) testing led to the present identification of surface membrane markers. Relating MAbs to cell surface antigens now provides a method for classifying and identifying specific cellular membrane characteristics. The current method of testing uses flow cytometry with immunofluorescence.



- Immunologic disorders can be divided into primary, secondary (acquired), and those mediated through immune mechanisms. Diseases associated with a primary immunodeficiency are comprised of 40% T cell disorders, 50% B cell disorders, 6% phagocytic abnormalities, and 4% complement alterations. The most common T cell deficiency states are those associated with a concurrent B cell abnormality.

REVIEW QUESTIONS

1. A function of the cell-mediated immune response not associated with humoral immunity is:
   a. Defense against viral and bacterial infection
   b. Initiation of rejection of foreign tissues and tumors
   c. Defense against fungal and bacterial infection
   d. Antibody production

2. The primary or central lymphoid organs in humans are the:
   a. Bursa of Fabricius and thymus
   b. Lymph nodes and thymus
   c. Bone marrow and/or fetal liver and thymus
   d. Lymph nodes and spleen

3. All the following are a function of T cells except:
   a. Mediation of delayed-hypersensitivity reactions
   b. Mediation of cytolytic reactions
   c. Regulation of the immune response
   d. Synthesis of antibody

4-7. Match the type of lymphocyte with its function (use an answer only once).

4. _____ T cells
5. _____ B cells
6. _____ K-type lymphocytes
7. _____ Natural killer (NK) cells
   a. Antibody-dependent, cell-mediated cytotoxicity (ADCC) reaction
   b. Cellular immune response
   c. Cytotoxic reaction
   d. Humoral response
   e. Phagocytosis

8-10. Match the surface membrane marker with the appropriate normal T cell type.

8. _____ CD4
9. _____ CD8
10. _____ CD3
    a. All or most T lymphocytes
    b. Helper-inducer T cells
    c. Suppressor-cytotoxic T cells

11. All the following are B cell surface membrane markers except:
    a. sIg
    b. Fc receptor
    c. C3 receptor
    d. CD4

12-17. Match the following congenital or acquired disorders with the major type of lymphocyte affected.

12. _____ Thymic hypoplasia
13. _____ AIDS
14. _____ Chronic lymphocytic leukemia
15. _____ Systemic lupus erythematosus
16. _____ Multiple myeloma
17. _____ Bruton's agammaglobulinemia
    a. Congenital T cell disorder
    b. Congenital B cell disorder
    c. Acquired T cell disorder
    d. Acquired B cell disorder

18. Most diseases associated with a primary defect are _____ disorders.
    a. T cell
    b. B cell
    c. Complement
    d. Phagocytic

19. Severe combined immunodeficiency is caused by:
    a. T cell depletion
    b. B cell depletion
    c. Inappropriate development of stem cells
    d. Phagocytic dysfunction

20. DiGeorge's syndrome is caused by:
    a. Faulty embryogenesis
    b. Deficiency of calcium in utero
    c. Inappropriate stem cell development
    d. Autosomal recessive disorder

21. The major clinical manifestation of a B cell deficiency is:
    a. Impaired phagocytosis
    b. Diminished complement levels
    c. Increased susceptibility to bacterial infections
    d. Increased susceptibility to parasitic infections

22. Bruton's agammaglobulinemia is a(n):
    a. Acquired disorder
    b. Autosomal genetic disorder
    c. Sex-linked genetic disorder
    d. Disorder occurring primarily in girls

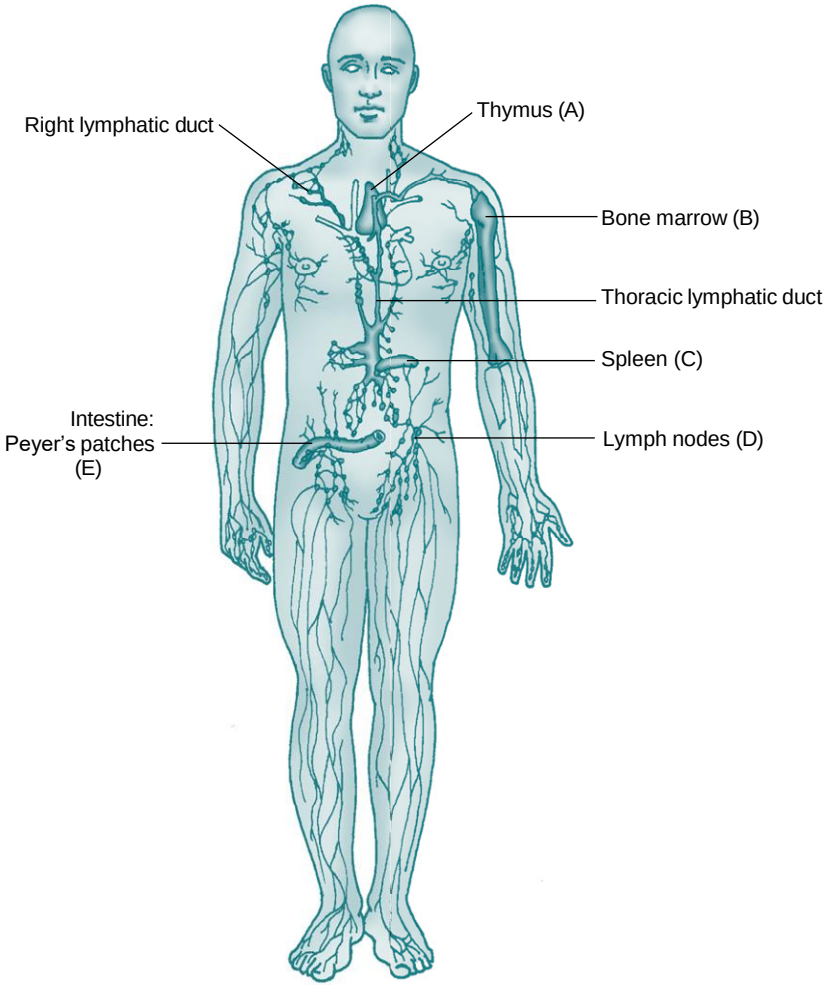
23. Which of the following disorders does not result in a secondary immunodeficiency?
    a. Sickle cell disease
    b. Uremia
    c. AIDS
    d. Poison ivy hypersensitivity

24. The secondary lymphoid tissues in mammals are:
    a. Thymus and bursa of Fabricius
    b. Lymph nodes
    c. Spleen
    d. Both b and c

25. In mammalian immunologic development, the precursors of lymphocytes arise from progenitor cells of the:
    a. Yolk sac
    b. Lymph nodes
    c. Spleen
    d. Both b and c

26. The thymus is embryologically derived from the:
    a. Yolk sac
    b. Pharyngeal pouches
    c. Lymphoblasts
    d. Bone marrow

27. Identify the sites of secondary tissue on the body diagram.
    a. A, B, and C
    b. B, C, and D
    c. B, D, and E
    d. C, D, and E



See question 31. *(Adapted from Turgeon ML: Clinical hematology: theory and procedures, ed 4, Philadelphia, 2005, Lippincott Williams & Wilkins.)*

28. The process of aging causes the thymus to:
    a. Decrease in size
    b. Not change over time
    c. Lose cellularity
    d. Both a and c

29. T lymphocytes can also be referred to as:
    a. Mast cells
    b. Memory cells
    c. Phagocytic cells
    d. Short-lived cells

30. Which of the following characteristics of T lymphocytes is false?
    a. Can form a suppressor-cytotoxic subset
    b. Can be helpers-inducers
    c. Can be CD4+ or CD8+
    d. Can synthesize and secrete immunoglobulin

31. and 32. Match each type of lymphocyte to the appropriate function.

31. _____ T lymphocytes

32. _____ B lymphocytes
    a. Cellular immune response
    b. Humoral antibody response

33-35. Match each to the appropriate function.

33. _____ Cytotoxic or effector T cells

34. _____ Helper or regulator T cells

35. _____ Suppressor T cells
    a. Secrete a variety of cytokines
    b. Recognize antigens associated with MHC class I
    c. Inhibit response of helper T cells

36. Natural killer cells:
    a. Produce interferon
    b. Produce IL-2
    c. Were previously called null cells
    d. All of the above

37. K-type cells:
    a. Synthesize antibody
    b. Secrete antibody
    c. Destroy by cytotoxic reaction
    d. Phagocytize target cells

38-41. Complete the chart, choosing from the following answers for the appropriate procedures:
    a. Screening for anti-A and anti-B isoagglutinins
    b. Erythrocyte sedimentation rate
    c. Absolute neutrophil count
    d. Absolute lymphocyte count

Initial Evaluation of Suspected Immunodeficiency

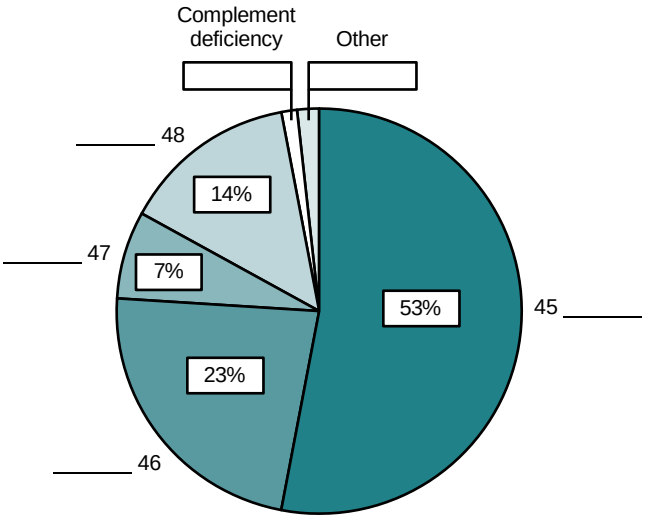
Suspected Immunodeficiency	Appropriate Laboratory Procedures
All suspected deficiencies	38. _____ Complete blood count with platelet evaluation
Antibody deficiency	39. _____ Screening for antibodies to diphtheria or tetanus toxoids
T cell deficiency	40. _____ Intradermal skin test
Phagocytic cell deficiency	41. _____

42. Calculate the absolute lymphocyte count when the following conditions exist:
    Total leukocyte count = 20×10^9/L
    Relative percentage of lymphocytes = 50%
    a. 5×10^9/L
    b. 10×10^9/L
    c. 15×10^9/L
    d. 20×10^9/L

43-44. Select an appropriate B cell disorder for each category (may use an answer more than once):
    a. Chronic lymphocytic leukemia
    b. Bruton's agammaglobulinemia
    c. Autoimmune disorder

T Cell Disorder	B Cell Disorder
Congenital	
DiGeorge's syndrome	43. _____
Acquired	
Hodgkin's disease	44. _____
Systemic lupus erythematosus	Systemic lupus erythematosus

45-48. Identify the distribution of immunodeficiencies shown in the illustration.



    a. T cell disorder
    b. B cell disorder
    c. Severe combined immunodeficiencies (SCIDs)
    d. Disorders of phagocytosis

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