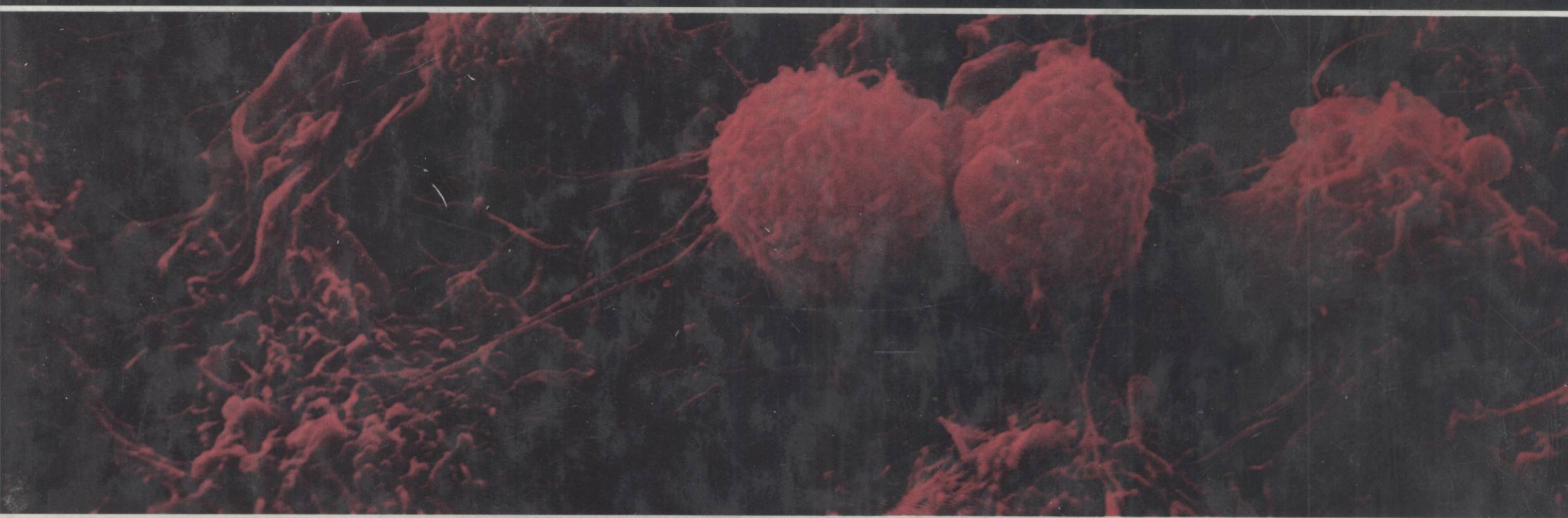
IMMUNOLOGY AND SEROLOGY IN LABORATORY MEDICINE



MARY LOUISE TURGEON

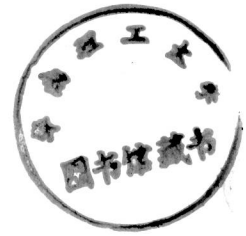
IMMUNOLOGY AND SEROLOGY IN LABORATORY MEDICINE

MARY LOUISE TURGEON
Ed.D., M.T. (ASCP)

Assistant Director of Medical Education
Guthrie Medical Center
Sayre, Pennsylvania

Research Assistant Professor
College of Medicine
State University of New York
Health Science Center at Syracuse
Syracuse, New York

Assistant Clinical Professor
University of North Dakota
School of Medicine
Grand Forks, North Dakota



With 107 illustrations

THE C. V. MOSBY COMPANY
St. Louis • Baltimore • Philadelphia • Toronto 1990

Editor: Stephanie Bircher
Assistant Editor: Anne Gunter
Production Editor: Radhika Rao Gupta
Designer: Susan E. Lane

Cover Photo: Macrophages, red blood cells and T-lymphocytes (2500×)
© Manfred Kage/Peter Arnold, Inc.

Printed in the United States of America

The C. V. Mosby Company
11830 Westline Industrial Drive, St. Louis, Missouri 63146

Library of Congress Cataloging-in-Publication Data

Turgeon, Mary Louise.
Immunology and serology in laboratory medicine.
Includes bibliography references.
1. Serum diagnosis. 2. Serology. 3. Immunology.
I. Title. [DNLM: 1. Allergy and Immunology—laboratory manuals. 2. Immunologic Technics. 3. Immunologic tests. 4. Serology—laboratory manuals. QW 525 T936i]
RB46.6.T87 1990 616.07'9 89-13448
ISBN 0-8016-5131-X

C/VH/VH 9 8 7 6 5 4 3

IMMUNOLOGY AND SEROLOGY IN LABORATORY MEDICINE

Preface

Immunology and Serology in Laboratory Medicine has been written primarily for undergraduate students in a clinical laboratory science program. This book is intended to fulfill the needs of medical laboratory technology (clinical laboratory technician—CLT) and medical technology (clinical laboratory science—CLS) students, and their instructors for an entry-level text that encompasses theory, practice, and clinical applications in the fields of immunology and serology. Practicing medical technologists, medical students and medical residents, nursing students and practitioners, students and practitioners in other allied health disciplines such as medical assisting, physician assistants, and practicing physicians such as general internists and family medicine specialists can use this text as a reference.

The purpose of this book is to describe the basic theoretic concepts in immunology, to explain the underlying theory of procedures performed in immunology and serology laboratories, to summarize clinical features of relevant selected disorders, and to detail procedures applicable to specific disorders. The major topical areas are organized into four primary sections. The initial two sections progress from basic immunologic mechanisms and serologic concepts to the theory of laboratory procedures including automated techniques. The latter two sections emphasize medical applications. The latter sections contain representative disorders of infectious and immunologic origin as well as topics such as transplantation and tumor immunology.

The sequence of the sections is designed to accommodate the core needs of clinical laboratory technology and clinical laboratory science students in basic concepts, the underlying theory of procedures, and immunologic manifestations of infectious diseases. Because the needs of medical technology students are more advanced in the area of immunopathology, these topics are presented in the latter part of the book in order to allow students to analyze and evaluate abnormalities based upon their knowledge of the preceding sections. Students may study specific components of the book depending upon the length and objectives of the course.

In order to achieve clarity, a topical outline is 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. Illustrations, photographs, and summary tables are used to visually clarify various conceptual themes and arrange detailed information. Chapter highlights and multiple-choice licensure-type review questions are provided at the conclusion of each chapter.

Procedures are organized according to the format suggested by the National Commission for Clinical Laboratory Standards (NCCLS). This format introduces students to the typical procedural write-up encountered in a working clinical laboratory. This format includes a brief statement of procedural theory and purpose; specimen requirements, handling and storage; reagents, supplies and equipment; quality control requirements; procedural steps; calculations; normal values; procedural notes, including sources of error, clinical applications, and limitations of the procedure; and procedural references.

The field of immunology has exploded with new information over the past decade. For example, two diseases (AIDS and Lyme disease) have been discovered or more fully described during the research and writing phases of this book. The subspecialty areas within the field have proliferated to the extent that a substantial body of knowledge unique to each particular subspecialty exists. Most current texts attempt to comprehensively include this enormous body of theoretic knowledge into a single book. Consequently, most books begin at an advanced level and fail to recognize the needs of the novice for basic information as well as medical applications that are of particular importance to students in the health professions. A few textbooks are more simplistic in presentation but are actually serology texts with limited immunology content.

Immunology and Serology in Laboratory Medicine has been written for beginning students in immunology, who need an emphasis on the medical aspects of the discipline. No attempt has been made to replace books written at more sophisticated reference levels. This text should provide students with a basic foundation in the theory and practice of clinical immunology during a one-term course.

Mary L. Turgeon

Acknowledgments

My objective in writing *Immunology and Serology in Laboratory Medicine* was to integrate basic science concepts and procedural theory in immunology and serology with relevant medical applications. Because of the rapidly expanding body of knowledge in immunology, writing a book that addresses the holistic needs of those in the clinical sciences has been a challenge. In addition, this book has provided me with the opportunity to share my experience and insight as a medical educator with others.

I would like to express gratitude to Rodney F. Hochman, M.D., Associate in Rheumatology, Guthrie Clinic/Guthrie Medical Center, Sayre, Pennsylvania; Steven Villaneuwava, M.D., Internal Medicine Resident, Robert Packer Hospital/Guthrie Medical Center, Sayre, Pennsylvania; and James A. Terzian, M.D., Chief Pathologist, St. Joseph's Hospital, Elmira, New York, who generously gave of their time to review portions of the manuscript. In addition, I appreciate the efforts of the members of the editorial and production staff at the C.V. Mosby Company.

Finally, special thanks to Don Turgeon for his forbearance during the period of manuscript preparation, and for once again contributing to the artistic elements of this, my third book.

To
Don
for his unwavering support of my pursuits

Contents

PART

I Basic Immunologic Mechanisms

CHAPTER 1

An Overview of Immunology

Immunology is defined as the study of the molecules, cells, organs, and systems responsible for the recognition and disposal of foreign (nonself) material, of how body components respond and interact, of the desirable and undesirable consequences of immune interactions, and of the ways in which the immune system can be advantageously manipulated to protect against or treat diseases. Immunologists in the Western Hemisphere generally exclude from the study of immunology the relationship between cells during embryonic development.

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 in all living organisms. Nonself substances can be as diverse as life-threatening infectious microorganisms or a lifesaving organ transplant. 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 (a condition in which the body's own tissues are attacked as if they were foreign).

BODY DEFENSES: RESISTANCE TO MICROBIAL DISEASE

Before a pathogen can invade the human body, it must overcome the resistance provided by the body's immune system, which consists of nonspecific and specific defense mechanisms (Fig. 1-1).

First Line of Defense

The *first line of defense* or first barrier to infection is unbroken skin and mucosal membrane surfaces. These surfaces are of utmost importance 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 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 contained in sweat possess antimicrobial properties. The production of ear wax is another example of a process that guards the auditory canals of the ear from infectious disease. Secretions produced in the process of eliminating liquid and solid wastes (e.g., the 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

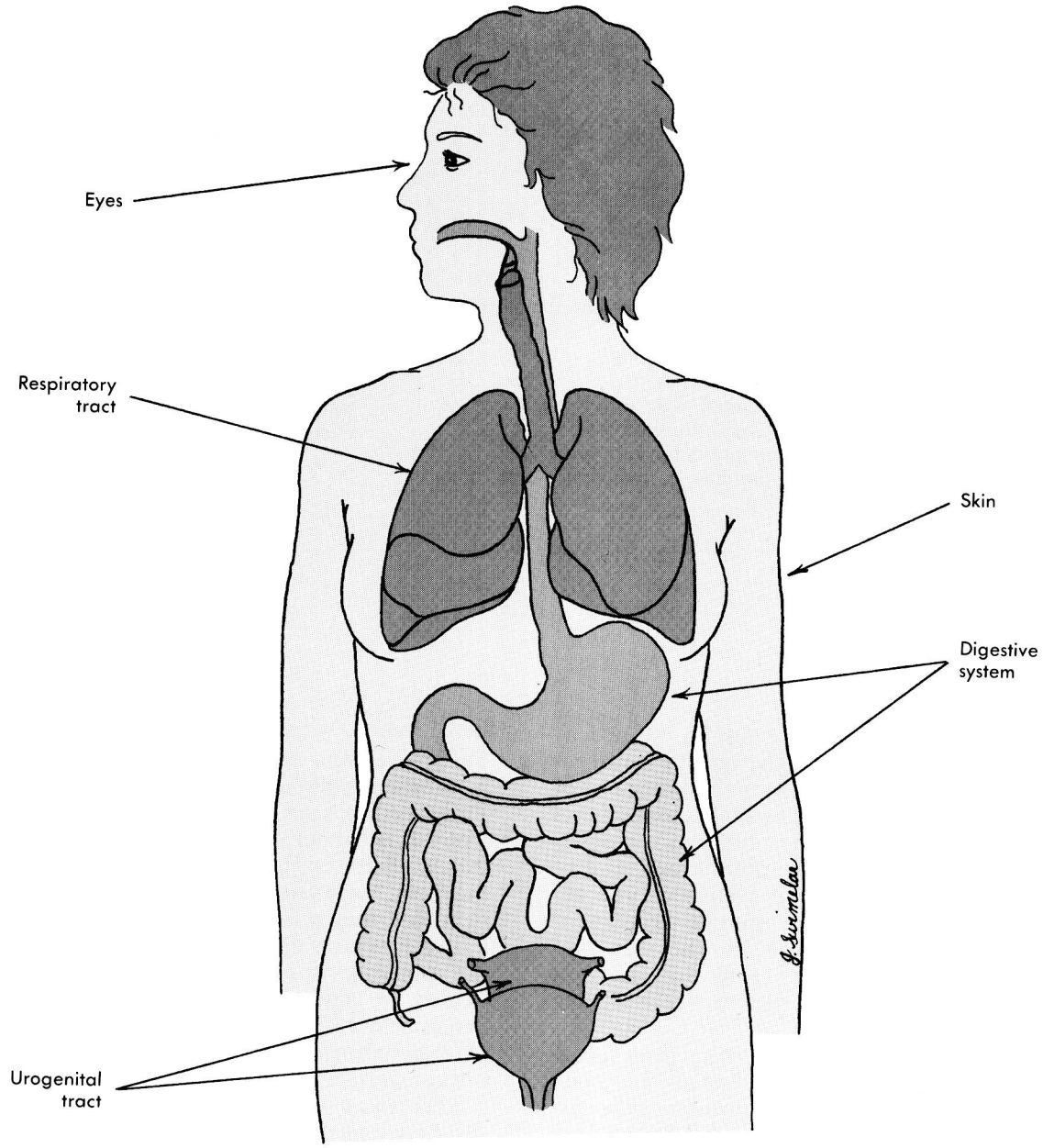


FIGURE 1-1

Natural body defenses. Body fluids, specialized cells, fluids, and resident bacteria (normal flora) allow systems such as the respiratory, digestive, urogenital, and integumentary systems to naturally defend the body against microbial infection.

potential pathogens, tears and saliva also have chemical properties that are of value in defending the body. The enzyme, *lysozyme,* is found in tears and saliva. Lysozyme attacks and destroys the cell wall of susceptible bacteria, particularly certain gram-positive bacteria. *IgA antibody* is another protective substance of importance in tears and saliva.

Thus the body has a wide variety of barrier-assisting defenses that protect against disease as the first line of defense. Although these barriers vary between individuals, they do assist in the general resistance to infectious organisms.

Natural Immunity

Natural (innate or inborn) *resistance* is one of the two ways that the body resists infection after microorganisms have penetrated the first line of resistance. The second form, *acquired resistance,* which specifically recognizes and selectively eliminates exogenous (or endogenous) agents, is discussed later in this chapter.

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 (see box below, left) becomes operational. The elements of natural resistance include *phagocytic cells, complement,* and *acute inflammatory reaction.* Despite their relative lack of specificity, these components are essential because they are largely responsible for natural immunity to many environmental microorganisms. Phagocytic cells (see Chapter 3), which engulf invading foreign material, constitute the major cellular component. Complement proteins (see Chapter 5) are the major humoral (fluid) component of natural immunity. Other substances of the humoral component are lysozymes and *interferon,* which are sometimes described as "natural antibiotics." Interferon is a family of proteins produced rapidly by many cells in response to viral infection. It functions to block the replication of virus in other cells.

Tissue damage produced by infectious or other agents results in inflammation, a series of biochemical and cellular changes that facilitate the *phagocytosis* (the engulfing and destruction) of microorganisms or damaged cells. 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 (see Chapter 3) are sensitive indicators of the presence of inflammatory disease and are especially useful in monitoring such conditions.

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. It 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 frequently leaves the host with 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, like natural immunity, is composed of cellular and humoral components (see box below, right). The major cellular component of this mechanism 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. The majority of the actions of the two categories of the adaptive response, humoral-mediated and cell-mediated immunity (Table 1-1), are exerted by the interaction of antibody with complement and the phagocytic cells of natural immunity, and of *T cells* with *macrophages.*

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 of antibodies to these substances are the specific meaning of immunity. *Antibody-mediated immunity* to infection is acquired if the antibodies are formed by the host or received from another source. These two types of acquired immunity (Table 1-2) are called *active* and *passive* immunity, respectively.

Active immunity can be acquired by natural exposure in response to an infection or natural series

TABLE 1-1

Characteristics of Humoral- and Cell-Mediated 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

Components of the Natural Immune System

Cellular	Mast cells Neutrophils Macrophages
Humoral	Complement Lysozyme Interferon

Components of the Adaptive Immune System

Cellular	T lymphocytes B lymphocytes Plasma cells
Humoral	Antibodies Lymphokines

TABLE 1-2

Comparison of the 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.

of infections, or it may be acquired by an intentional injection of an antigen. This intentional injection of antigen, *vaccination,* is an effective method of stimulating antibody production and memory (acquired resistance) without suffering from the disease. Suspensions of antigenic materials used for immunization are varied and may be of animal or plant origin. These products may be composed of living suspensions of weak or attenuated cells or viruses, killed cells or viruses, or extracted bacterial products such as the 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* (a host that is able to recognize a foreign antigen and build specific antigen-directed antibodies) and cause permanent antigenic memory. Booster vaccinations may be needed in some cases to expand the pool of memory cells. The mechanism of antigen recognition and antibody production is discussed in Chapter 2.

Artificial passive immunity is achieved by infusion of serum or plasma containing high concentrations of antibody. This form of passive immunity provides immediate antibody protection against microorganisms, such as hepatitis A, by administering preformed antibodies. These antibodies have been produced by another person or animal that has been actively immunized, but the ultimate recipient has not produced them. The recipient will only temporarily benefit from passive immunity for as long as the antibodies persist in their circulation.

In addition, passive immunity can be acquired naturally by the fetus because of the transfer of antibodies by the maternal circulation in utero. Maternal antibodies are also transferred to the newborn after parturition in the prelactation fluid, *colostrum.* In order for the newborn to have lasting protection, active immunity must occur.

Immediate hypersensitivity comprises a subset of the body's antibody-mediated mechanisms. This subset consists of the reactions primarily mediated by *immunoglobulin E (IgE),* a class of immunoglobulins with unique biologic properties. Expression of immediate hypersensitivity results from the following:

1. Exposure to antigen (allergens)
2. Development of an IgE antibody response to the antigen
3. Binding of the IgE to mast cells
4. Reexposure to the antigen
5. Antigen-interaction with antigen-specific IgE bound to the surface membrane of mast cells
6. Release of potent chemical mediators from sensitized mast cells
7. Action of these mediators on various organs

Atopic diseases are processes mediated by or related to IgE-immediate hypersensitivity. The most dramatic and devasting systemic manifestation of immediate hypersensitivity is *anaphylaxis.* Anaphylaxis is an immediate (type I) hypersensitivity reaction characterized by local reactions, such as *urticaria* (hives) and *angioedema* (redness and swelling), or systemic reactions in the respiratory tract, cardiovascular system, gastrointestinal tract, or skin. This type of reaction can be fatal. Other types of atopic diseases include allergic rhinoconjunctivitis, urticaria, angioedema, asthma, gastrointestinal allergy, and atopic dermatitis, an eczematous skin eruption.

In addition to IgE-dependent hypersensitivity, two other immunoglobulin-dependent (antibody-dependent) mechanisms and a fourth, cell-mediated, delayed hypersensitivity mechanism exist. These clinical reactions are discussed in detail in Chapter 23. An alternate system of classification for hypersensitivity was developed by Gell and Coombs over two decades ago. Characteristics of this classification of hypersensitivity are presented in Table 1-3.

Cell-Mediated Immunity

Cell-mediated immunity consists of immune activities that differ from antibody-mediated immunity (Table 1-1). Cell-mediated immunity is moderated by the link between T lymphocytes and phagocytic cells, i.e., *monocytes-macrophages.* Lymphocytes (T cells) do not recognize the antigens of microorganisms or other living cells such as *allografts* (a graft of tissue from a genetically different member of the same species, e.g., a human kidney) directly, but rather when the antigen is present on the surface of an antigen-presenting cell, the macrophage. Lymphocytes are immunologically active through various types of direct cell-to-cell contact and by the production of soluble factors, *lymphokines,* for specific immunologic functions such as the recruitment of phagocytic cells to the site of inflammation. The roles of various types and subset

TABLE 1-3

Classification of Hypersensitivity Reactions

	Type I	Type II	Type III	Type IV
	Anaphylactic	Cytotoxic	Immune complex	T-cell dependent
Antibody	IgE	IgG Possibly other	IgG IgM	None
Complement Involved	No	Yes	Yes	No
Cells Involved	Mast cells Basophils	Red cells White cells Platelets	Host tissue cells	T cells Macrophages
Examples	Anaphylaxis Hay fever Food allergy	Transfusion reactions Hemolytic disease of newborn Thrombocytopenia	Arthus reaction Serum sickness Pneumonitis	Allergy of infection Contact dermatitis

Modified from Barrett JT: Textbook of immunology, St Louis, 1988, The CV Mosby Co.

types of lymphocytes are discussed in Chapter 4.

The term *delayed hypersensitivity* is often used synonymously with the term *cell-mediated immunity. Delayed hypersensitivity,* however, refers to the slow appearance of a secondary response in the skin and dates back to the time when antibody responses were detected by immediate hypersensitivity and reflected the subtle difference in the length of time that it took for a delayed response to occur (e.g., tuberculin skin test). Cell-mediated immunity is responsible for the following immunologic events:

1. Contact sensitivity (e.g., poison-ivy dermatitis caused by binding of substance to the skin)
2. Delayed hypersensitivity (e.g., contact dermatitis)
3. Immunity to viral and fungal antigens
4. Immunity to intracellular organisms
5. Rejection of foreign-tissue grafts
6. Elimination of tumor cells bearing neoantigens
7. Formation of chronic granulomas (undegradable material such as tubercle bacilli, streptococcal cell walls, asbestos, or talc, sequestered in a focus of concentric macrophages that also contains some lymphocytes and eosinophils)

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

FACTORS ASSOCIATED WITH IMMUNOLOGIC DISEASE

Many of the same factors, such as general health and the age of an individual, are important in the development of immunologic as well as infectious disease. In the case of noninfectious diseases or disorders, however, additional factors may be of importance. These factors can include genetic predisposition to many disorders, nutritional status, and the individual's method of coping with stress.

Effect of Age on Immunity

Although nonspecific and specific body defenses are present in the unborn and newborn infant, many of these defenses are not completely developed in this group. Therefore young children are at greater risk for diseases, particularly infectious diseases.

A loss of immune defenses, not disease itself, may be the cause of death in at least 30% of people over 85 years of age. In the elderly, certain natural barriers to infection break down. Changes in the skin due to the normal aging process allow it to be breached more readily. In the lung, many of the specialized defenses against foreign invasion are weakened, including the cough reflex and bronchotracheal ciliary action. Other age-related changes include incomplete emptying of the bladder that can lead to infection and alteration in the normal flora of the intestine, caused by immobilization or as a result of drug therapy. In addition, some age-associated diseases exert detrimental effects on the immune system. Diabetes, which is increasing in incidence in older persons, results in greater susceptibility to diseases such as septicemia and gangrene.

The ability to respond immunologically to disease is age related. It has been suggested that faulty immunologic reactions are involved in the aging process; however, the effect of aging on the immune response is highly variable. In studies of the cells of the immune system, a general decline in the quantity of some types of lymphocytes in the blood has been observed in some elderly persons. A decrease in lymphocyte subset types and aberrant functioning of immunoregulatory cells have been implicated as potential causes of many age-related immunologic dysfunctions that contribute to poor

immunity in the aged. It is not known if enhancement of the immune response with methods such as tissue removal, dietary manipulation, cell grafting, and chemical intervention in the elderly will be associated with clinical benefits; but immunomodulation may be a formidable tool to combat aging of the immune system in the future.

Role of Nutrition and Immunity

The importance of good nutrition to good health has always been emphasized. Good nutrition is known to be important to growth and development, and it is now suggested that a healthy diet is important in the aging process and in the triad of nutrition, immunity, and infection. The consequences of diet, however, in many aspects of the immune response have been documented in multiple disorders. Every constituent of body defenses, including phagocytosis and humoral and cellular immunity, appears to be influenced by nutritional intake. Deficient or excessive intake of some dietary components, such as vitamins and minerals (Table 1-4), can exert negative effects on the immune response. Therefore a healthy diet is important to maximum functioning of the immune system.

Effects of a Proper Diet

The study of the relationship of nutrition to immunity is complex because of factors such as the diversity of the food we eat and the influence of environment on specific nutritional needs. Some nutritional associations hold true for the risk of malignancy and immune function but others do not. The role of nutrition in the risk and treatment of malignancy has been studied. For example, low intake of vitamin A and high intake of fats have been associated with an increased risk of malignancy in humans. Both constituents also have a marked effect on the immune response. It has been suggested that the balance and absolute intake of multiple nutrients have an influence on susceptibility to in-

TABLE 1-4

Examples of the Effects of Increased or Decreased Levels of Vitamins and Minerals

Constituent	Effect
Water-soluble vitamins	
Folic acid	Deficiency has a profound effect on cell-mediated immunity.
Pantothenic acid	A deficiency in conjunction with pyridoxine deficiency is associated with the absence of antibodies.
Vitamin B_1 (Thiamin)	Deficiency can produce abnormal phagocytosis, e.g., Schwachman-Diamond syndrome.
Vitamin B_2 (Riboflavin)	No disease associated with deficiency. Specific role in human malignancy is unclear but believed to play a role in tumorigenesis.
Vitamin B_6 (Pyridoxal, pyridoxine)	Deficiency during prenatal and postnatal development affects organs of the immune system, spleen, and thymus respectively. Deficiency in children and adults can cause mild impairment, e.g., decreased lymphocytes; decreased hormones produced by immune organs (e.g., thymus); inability to produce antibodies to various antigens; and depression of delayed hypersensitivity. If simultaneous deficiency in pantothenic acid, complete absence of antibody production will occur.
Water-soluble vitamins—cont'd	
Vitamin B_{12} (Cobalamin)	Congenital deficiency of transcobalamin II,* associated with decreased white cells; the absence of immunoglobulins; impaired phagocytosis.
Vitamin C (Ascorbic acid)	Increased or decreased amounts may negatively affect phagocytosis.
Fat-soluble vitamins and congeners	
Vitamin A	Decreased intake of vitamin A and a high intake of fats is associated with an increased risk of malignancy.
Minerals	
Cadmium Lead	Excess but subtoxic amounts have a negative effect on normal immune function.
Copper	Deficiency associated with increase in severity of inflammatory lesion and antibody-forming cell response.
Iodine	In excess has a dose-dependent immunosuppressive effect.
Iron	Deficiency probably increases susceptibility to infection because iron is an integral part of microbicidal process.
Selenium	Deficiency impairs T cell–dependent antibody responses, particularly in association with vitamin E deficiency.

*Proteins that deliver vitamin B_{12} to the tissues.