LABORATORY IMMUNOLOGY AND SEROLOGY

Neville J. Bryant

W.B. SAUNDERS COMPANY
PHILADELPHIA LONDON TORONTO
MEXICO CITY RIO DE JANEIRO
SYDNEY TOKYO HONG KONG

1986

LABORATORY IMMUNOLOGY AND SEROLOGY

SECOND EDITION

Neville J. Bryant, ART, FACBS
Technical Director, Serological Services, Ltd., Toronto
President, Serological Serums, Ltd.
Toronto, Ontario, Canada

W. B. Saunders Company: West Washington Square
Philadelphia, PA 19105

Library of Congress Cataloging in Publication Data

Bryant, Neville J.
Laboratory immunology and serology.

Includes bibliographies and index.
1. Serology—Technique. 2. Immunodiagnosis.
I. Title. [DNLM: 1. Allergy and immunology—laboratory manuals. 2. Serology—laboratory manuals. QW 525 B915L]

RB46.5.B79 1986 616.07'561 85-11871

ISBN 0-7216-1059-5

Editor: Baxter Venable
Designer: Bill Donnelly
Production Manager: Laura Tarves
Illustration Coordinator: Peg Shaw

Laboratory Immunology and Serology ISBN 0-7216-1059-5

Last digit is the print number: 9 8 7 6 5 4 3 2 1

PREFACE

This book is designed for students of medical technology studying the sciences of serology and immunology as a separate entity, or as part of a microbiology course. The material provides the theory and practical procedures necessary for a basic yet thorough understanding of serological principles.

In response to readers' requests, the immunology section of the book in this, the second edition, has been greatly enlarged. The material presented, however, remains at an introduction level, unencumbered by the research information which is of little use to the student.

Again, each chapter is preceded by a set of learning objectives and each contains a set of review questions with answers given at the end of the book. General references are given after each chapter that will direct the student to other texts containing specific information about the subject under discussion.

The book attempts to say nothing startling or new; its purpose is to introduce the student to the fascinating science of serology and immunology and, perhaps, to whet the student's appetite for further, more advanced study.

Many people have contributed in one way or another to the preparation of the second edition, and I wish to express my gratitude to them. Most especially, my thanks go to Nancy Harrison, who performed many of the tasks necessary to achieve the completion of the text with diligence and cheerfulness.

Appreciation is also due to the people at W. B. Saunders Company, who are always supportive and patient—most especially Baxter Venable, my editor, whose interest in this project is always there, and who has shown patience, support and inspiration during the lengthy preparation of this second edition.

NEVILLE J. BRYANT

CONTENTS

ONE

INTRODUCTION: NONSPECIFIC (NATURAL, INNATE) IMMUNITY

OBJECTIVES

The student shall know, understand, and be prepared to explain:

1. A brief history of immunology
2. Types of nonspecific immunity
3. The concepts of susceptibility and nonsusceptibility
4. The role of the epithelial barriers in nonspecific immunity
5. Inflammation, with special emphasis on the vascular and cellular response
6. Phagocytosis, encompassing the contributing mechanisms: chemotaxis, opsonization, ingestion, and degranulation
7. The concepts of nonspecific humoral immunity with respect to:
   a. "Natural antibody"
   b. Lysozyme
   c. Properdin
   d. Betalysin
   e. Interferon
   f. Complement

The science of immunology represents that area of biology that is concerned with the processes by which all living organisms (including human beings) defend themselves against infection (*i.e.*, the study of immunity).

The term *immunity* can be used to imply "resistance" in its broadest sense, including resistance to infectious agents, foreign particles, toxins (poisonous substances), living cells, and cancer.

The principles of immunology stem almost from the earliest written observations of humankind, in which it was noted that individuals who recovered from a certain disease rarely contracted the same disease again. This observation prompted deliberate attempts to induce immunity: In A.D. 1500, the Chinese developed a custom of inhaling crusts from smallpox lesions to prevent the development of smallpox in later life. The procedure was, at best, hazardous. By 1718, the practice of injecting material from crusts or fluids from smallpox blisters (known as "variolation") was used extensively throughout the Eastern world and was introduced into Western medicine by Lady Montagu, the wife of the British ambassador to Turkey, who had her children so treated. By the time that the American colonies developed into an independent nation, variolation was a reasonably common practice, and clear evidence had been obtained that it was effective in most cases. The problem that could not be overcome, however, was that the virus used could be transmitted; therefore, protection by variolation was hazardous to the community at large.

In 1798, an English physician, Jenner, published his monumental work on vaccination, describing a

related, yet safe, procedure. Realizing that individuals who had had cowpox were spared in smallpox epidemics, he inoculated a boy with pus from an individual who had cowpox and subsequently reinoculated the same boy with infectious pus from a patient in the active state of smallpox. No disease state followed these inoculations, and the experiment was repeated several times with great success. The term *vaccination* (L. *vacca* = cow) was applied to the procedure and referred specifically to the injection of smallpox "vaccine." The term has now come to mean any immunizing procedure in which vaccine is injected.

Jenner's discovery provided the first clear evidence that active immunization could be used safely to prevent an infectious disease, that attenuated (thinned, weakened) viruses could be used for effective active immunization, and that resistance to infection might be related to the speed and intensity of inflammatory reactions. The concept of interference of one virus infection by another was also suggested in Jenner's work.

Almost 70 years later, these discoveries were extended by Pasteur, who showed that heat could kill bacteria, and, from this observation, the term *pasteurization,* came into use. In addition, Pasteur recognized and exploited the general principles underlying vaccination through the observation that the inoculation of the causative agents of chicken cholera and anthrax in animals induced immunity against these diseases. Later, he also made the outstanding contribution of vaccination against rabies.

In the two decades before 1900, Elie Metchnikoff, a Russian biologist, who was one of the major pioneers of immunology, elucidated the role of phagocytosis and cellular immunity. Within the same period of time, killed vaccines were introduced, complement (alexin) was described, and the comparative roles of complement and cell lysis were elucidated.

Discoveries in the field of immunology during the twentieth century have been numerous and have profoundly influenced the development of every branch of medicine and surgery. In 1903, Write and Douglas demonstrated that acquired immunity resulted from both humoral and cellular elements and described opsonization. As a result of these observations and discoveries, the term *antigen* (antibody + Gr. *gennan* = to produce) came into regular use to describe the agent that conferred immunity on the host by the production of specific antibody.

In 1902, Richet and Portier provided evidence that the immune reaction could be damaging as well as beneficial by showing anaphylaxis to be an immunologic reaction. The following year, the Arthus reaction was described, and, at about the same time, von Pirquet and Schick showed as part of their studies of serum sickness that diseases of the skin, heart, joints, blood vessels, and kidneys, as well as fever, could be caused by the body's immunologic reaction to foreign protein. By 1920, the immunologic basis of certain kinds of allergy had also been reported by Prausnitz and Kustner.

In another direction of inquiry, Paul Ehrlich was the first to use quantitative measurements of immune reactions. Then, in 1928, Alexander Fleming discovered penicillin, and, in 1932, Gerhard Domagk developed prontosil, which was found to be an effective antibacterial agent against streptococcus. Later, the active ingredient was found to be sulfanilamide, which proved to be active against a wide variety of organisms.

The understanding of immune reactions has been enhanced by the techniques for analysis of these processes, including the precise chemical methods for measurement of antigens and antibodies through precipitin analysis (introduced by Heidelberger, 1924-1926), immunoelectrophoresis (Grabar and Williams, 1953), and so forth.

Studies of immunodeficiency and structure-function relationships in the lymphoid system were initiated as a result of the discovery of hypogammaglobulinemia (Burton, 1952), which eventually led to the dissection of the immune system into two separate areas, known as the T- and B-cell systems.

In addition, the studies of Medawar *et al.* (1944, 1945) initiated interest in transplantation and showed that the immunologic processes are clearly involved in allograft rejection of normal organs.

At the present time, it is safe to say that immunology has an impact on all medical disciplines. Many patients are recognized who have immunologic deficiencies or abnormal immune responses as the sole basis for their disease. It is therefore clear that no other body of knowledge is as important for medical personnel to study and understand as are the fundamentals of the immune process.

NONSPECIFIC IMMUNITY

Two types of immunity are recognized: (1) that which is present at the time of birth or that develops during maturation ("natural"), and (2) that which is acquired as a result of prior experience with a foreign substance.

Nonspecific ("natural" or "innate") immunity, therefore, is the process by which all animals (including humans) resist the invasion of foreign or potentially harmful microorganisms by natural means (*i.e.,* without the production of protective antibodies). This includes the concepts of susceptibility and nonsusceptibility, epithelial barriers, antibacterial agents, inflammation, complement,

phagocytosis, acid pH of stomach, normal intestinal flora, and so forth.

Susceptibility and Nonsusceptibility

Certain animal species are resistant to particular disease states, whereas other species are highly susceptible to them. This phenomenon is not clearly understood, although there is growing evidence that it is controlled to some extent by hereditary or genetic influences. An example of this would be the Fy^a and Fy^b (Duffy) receptors on the erythrocyte membrane, which are believed to be associated with susceptibility to malaria. Studies by Miller and colleagues (1975, 1976) showed that the red cells of individuals who had not inherited the Fy^a or Fy^b receptors (*i.e.,* individuals of Duffy phenotype Fy[a − b −]) were resistant to invasion *in vitro* with *Plasmodium knowlesi*. Of 17 volunteers exposed to the bites of *Plasmodium vivax*–infected mosquitoes, only those with the red cell phenotype Fy(a − b −) were resistant to erythrocyte infection.

The concepts of susceptibility and nonsusceptibility are not confined to species differences; they are evident among different races of humans and can be affected by age and the influence of hormones.

The Epithelial Barriers

The skin and mucous membranes provide the body with a physical barrier against invasion and, in addition, possess certain active mechanisms for the killing of bacteria and other organisms. This protection is in many ways remarkable, because many epithelial surfaces consist of only a single layer and are exposed to large numbers of bacteria. Complete sterilization of these surfaces by artificial means is impossible except, perhaps, for brief periods of time.

The self-sterilizing power of the skin is achieved by desiccation (drying), epithelial desquamation (shedding), pH, and, most important, the secretion of fatty acids that have antibacterial properties.

Mucosal surfaces, by contrast, are protected by a so-called slime layer, which has been shown to possess antibodies of the IgA class (see page 15) and other antimicrobial and antiviral substances. The bacterial enzyme *lysozyme* is present in abundance in such secretions (*e.g.,* saliva, tears), as well as in the granules of polymorphs and macrophages, and is widely distributed throughout the body fluids. This enzyme is especially effective in lysing certain bacteria (*e.g., Micrococcus lysodeikticus*).

In addition to this, a nonspecific antiviral agent known as *interferon*, which inhibits intracellular viral replication, is itself synthesized by cells in response to viral infection. It is evident that interferon is a major factor in the recovery from (as distinct from the prevention of) viral infections.

Inflammation

Inflammation is the term used to describe the condition into which tissues enter as a reaction to injury—the classic signs of which are pain, heat, redness, and swelling, and sometimes a loss of function. The inflammatory process involves the *cellular defenses* of the body, which are among the most efficient and adaptive of all mechanisms available for the resistance to invasion by parasitic microorganisms. In addition, the *vascular response* aids in preventing the invasion of bacterial agents beyond the periphery of the body.

In brief, the inflammatory process is characterized by the vascular response and the cellular responses.

The Vascular Response. The primary response in acute inflammation is the dilation of the artery so that more blood passes to the area of injury. This increased content of blood is termed *hyperemia* and is the reason why the inflamed area appears red. As a result of this, plasma leaks from the vessels, making the blood more viscoid; the lubricating action of the plasmatic zone is impaired; and the stream of blood slows down (referred to as *stasis*). At the same time, the endothelial cells become swollen, and the spaces between adjacent cells become widened, thereby permitting plasma and cells to pass between them.

The most characteristic feature of acute inflammation is the formation of an exudate that has both a fluid and a cellular component. The fluid exudate is formed as a result of increased vascular permeability, which allows the plasma proteins to leak through the vessel wall, causing the osmotic pressure effect of the plasma proteins to be lost. In addition, there is an alteration in the "ground substance," which becomes more fluid, thus allowing the exudate to diffuse into the surrounding tissues more readily, preventing an immediate rise in tissue tension. Although it is normal for the tissues to drive fluid back into the venules, tissue tension does eventually increase, thus limiting the amount of exudate formed and causing pain.

The fluid exudate has almost the same composition as plasma, and it contains antibacterial substances (*e.g.,* complement) as well as specific antibodies. Drugs and antibiotics, if present in the plasma, also appear in the exudate.

In addition to these effects, the fluid exudate serves to dilute any irritating chemicals and bacterial toxins that might be present. In addition, the fibrinogen that is in the exudate is converted to

fibrin by the action of tissue thromboplastins, and a fibrin clot forms. This fibrin forms a fine network of fibers, which provides a union between severed tissues, acts as a barrier against bacterial invasion, and aids phagocytosis (discussed later in this chapter).

The Cellular Response. The cellular response begins when white cells move into the plasmatic zone at the site of injury and stick to the altered vessel wall. At first, this adhesion is brief, after which the cells either roll gently along the endothelial lining or get swept back into the blood stream. Later, however, the cells adhere more firmly and line the endothelium, forming masses that may even block the lumen (known as *pavementation of the endothelium*). These adhering white cells will eventually push pseudopodia between adjacent endothelial cells, penetrate the basement membrane, and emerge on the external surface of the vessel (known as *emigration of the white cells*). The gap that is left by the emigrating white cells soon closes behind it, although sometimes a few red cells escape at the same time.

A characteristic feature of the cellular exudate is that in the initial stages of development, neutrophil polymorphonuclear leukocytes (polymorphs) predominate, but, as time goes by, these are replaced by monocytes (probably due to the faster migration and limited life span of polymorphs, which die off, leaving the long-lived mononuclear cells to replace them). Lymphocytes are also found in areas of inflammation, particularly during the healing process, although in certain instances (*e.g.*, viral infections, acute dermatitis), they are the predominant cell in the early stages of inflammation.

The function of the cellular exudate is to enact the process known as *phagocytosis*.

Phagocytosis

The action of the phagocytes is one of the most remarkable and fascinating of all body defense mechanisms. Early descriptions of the phenomenon known as phagocytosis are attributed to Elie Metchnikoff (1907), a Russian-born biologist, who recognized that specialized phagocytic (eating) cells provide a defense mechanism against invasion by engulfing foreign particulate matter, which they then attempt to destroy enzymatically. Metchnikoff observed that the process is a very general one and can be observed in animals of all stages of evolution.

The mechanisms contributing to phagocytosis include *chemotaxis, opsonization, ingestion,* and *degranulation.*

Chemotaxis. Chemotaxis is a process in which cells tend to move in a certain direction under the stimulation of chemical substances. This stimulation can cause two effects: (1) the cells may move *toward* the stimulating substance (known as *positive* chemotaxis), or (2) the cells may move *away* from the stimulating substance (known as *negative* chemotaxis). Without the influence of these chemotactic substances, cell motion is random.

Leukocytes have never been shown to display negative chemotaxis; they are always drawn *toward* the substance and, therefore, to the site of injury. This is a critical early step in phagocytosis.

A considerable amount of research has been devoted to the identification of the chemicals responsible for chemotaxis in acute inflammation in humans. Agents such as starch and certain bacteria have been shown to attract both polymorphs and monocytes *in vitro*. Other chemotactic agents are antigen-antibody complexes and dead tissue, although these function only if complement is present and activated ($\overline{C567}$ and the anaphylatoxins $\overline{C3}$a and $\overline{C5}$a being the chemotactic agents).

Opsonization. Of great importance in the phagocytic process is a group of antibodies known as serum opsonins (*i.e.*, antibodies and complement components). These antibodies interact with the surface of bacteria, rendering it acceptable to the phagocyte. Antibodies are able to opsonize by themselves, or they can cause the complement system (see page 24) to generate $\overline{C3}$ ($\overline{C1423}$), which coats the bacterium. Phagocytes apparently possess surface Fc receptors for Ig and $\overline{C3}$ receptors that recognize and interact with antibodies and activated $\overline{C3}$. Because time is required for these antibodies to develop, they are of greater significance in the later stages of inflammation than in the earlier stages.

Ingestion. Once the phagocyte has recognized that a particle is foreign, engulfment occurs by active ameboid motion (*i.e.*, resembling an ameba in movement). The phagocyte extends its cytoplasmic membrane around the invading organism, which is eventually surrounded and completely enclosed. This final structure containing the phagocytosed particle is known as the phagocytic "vacuole" or "phagosome" (Fig. 1–1).

Degranulation. When ingestion of the foreign particle is complete, cytoplasmic lysosomes (minute cell particles), which contain certain hydrolytic enzymes and peroxidase, approach the phagosome (vacuole), fuse with it, rupture, and discharge their contents into it. The mechanisms by which this phenomenon occurs are unknown. The cell then becomes degranulated as foreign materials (with the exception of inert materials) are digested. Bacteria are not always destroyed by hydrolytic enzymes. Some may survive and eventually break out of the cell again.

Nonspecific Immunity of Body Fluids

Body fluids and secretions have long been known to possess antibacterial properties. In many in

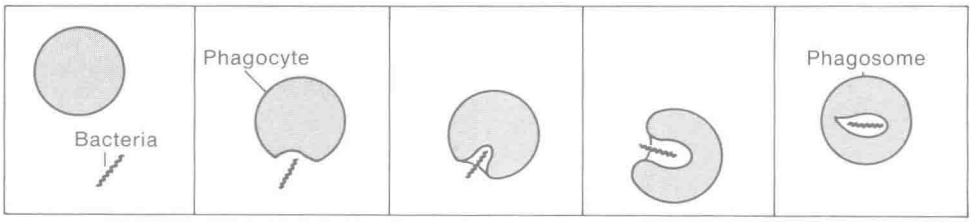

Figure 1–1. Phagocytosis (ingestion of foreign matter).

stances, the action of specific antibody and complement accounts for these properties; however, additional substances that play a significant role in nonspecific defense may be present.

"Natural" Antibody. An antibody that is present in a host and reacts with substances with which the host has had no known contact is referred to as "natural" antibody. Three possible mechanisms have been proposed to explain their formation:

1. *Genetic.* It is possible that natural antibody production is under genetic control and, therefore, that no antigenic stimulation of any kind is required for this production.
2. *Cross-reaction.* It has also been proposed that natural antibodies may be produced as a result of stimulation by specific antigens that have similar (but not identical) antigenic determinant groups that cross-react with the particular antigen by virtue of these chance similarities.
3. *Antigenic stimulation.* It is also possible that "natural" antibodies arise as the result of direct, specific antigenic stimulation—the antigens gaining access to the host by natural means. This possibility is the one that has achieved the widest general acceptance.

Lysozyme. Lysozyme is an enzyme found in many types of cells, which has been shown to have antibacterial activity. The enzyme functions by virtue of mucolytic properties that cleave acetylamino-sugars, the backbone of both gram-positive and gram-negative bacteria. Certain basic polypeptides (with large amounts of lysine) have been shown to kill anthrax. A synergistic action of the effect of lysozyme and complement has been observed in *in vitro* studies.

Properdin. Properdin is a serum protein that exerts bactericidal and viricidal effects in the presence of the third component of complement (C3) and magnesium ions. Originally described as a naturally occurring euglobulin with a broad spectrum of activity against bacteria, evidence now suggests that the presence of minute amounts of "natural" antibody accounts for the properdin effect. Properdin, however, is indistinguishable from these antibodies by virtue of the fact that they are capable of combining with certain monomolecular antigens to activate the complement sequence at the level of the third component (see Complement Activation—The Alternative Pathway, page 32).

Betalysin. The serum from many animal species contains a heat-stable substance with antibacterial activity. This substance (which is not present in plasma from the same animal) is known as betalysin. The substance is released during coagulation and probably functions through an enzymatic alteration of the cell surfaces of susceptible bacteria.

Interferon. Interferon is a nonspecific inhibitor of viral replication, its action being at the cellular level. It is a substance that has a molecular weight of about 25,000, which is released from infected cells following viral infection. It is also released from sensitized lymphocytes upon interaction with specific antigens and nonspecific stimulators (*e.g.*, phytohemagglutinins, PHA). Interferon is known to be significant in the recovery mechanism of viral infections, being particularly well suited to cell-mediated responses involving interactions *in vivo*.

Complement. Complement may be regarded as a group of nonspecific serum components, which, when activated by the interaction of antigen and antibody, combine in a fixed sequence and thereby enhance the effect of antibody (see Chapter 3).

The following is a brief summary of the biologic functions of complement in immune defense:

1. *Cytolysis.* A few species of bacteria are lysed by complement—primarily the gram-negative organisms, such as *Escherichia coli.* This is achieved through the full activation of the complement system, which allows lysozyme to reach the plasma membrane, where it destroys the mucopeptide layer.
2. *Immune Adherence* (C3b). This leads to the phagocytosis of microorganisms after coating with opsonizing antibody and complement or after complement activation via the alternative pathway (see page 32). Because many C3 molecules are bound to the surface membrane at each complement activation site, adherence to macrophages and polymorphs will occur more frequently through C3 than through IgG binding.
3. *Immunoconglutinin.* This may play a role in defense by agglutinating relatively small complexes containing bound C3, thereby making them more susceptible to phagocytosis.
4. *Inflammation.* The split fragments from complement components during consumption stimulate two helpful features of acute inflammation:

a. *Chemotactic factors.* By chemotaxis, phagocytic neutrophil polymorphs are attracted to the site of complement activation.
b. *Anaphylatoxin.* Anaphylatoxin, through histamine release, increases vascular permeability and hence the flow of serum antibody and complement to the infected area.

5. *Others.* Complement is also implicated in disease processes involving cytotoxic and immune complex–mediated hypersensitivities:
   a. *Cytotoxic reactions.* These are not uncommon findings in nephrotoxic nephritis and autoimmune hemolytic anemia.
   b. *Immune complexes.* These are formed in antibody excess, giving rise to immune vasculitis of the Arthus type. Soluble complexes formed in antibody excess give "serum-sickness"–type reactions.

In paroxysmal nocturnal hemoglobinuria (PNH), red cells are particularly sensitive to complement lysis due to an ability to fix the activated trimolecular complex $\overline{C567}$ at night when the pH of the plasma decreases. It should be noted that this is an erythrocyte membrane defect and is not due to an abnormality in the complement system.

Other Nonspecific Factors. Plant extracts, the sera of invertebrates, and other body fluids of animals have been shown to have antibacterial activity. Although the properties of most of these substances have not been extensively studied, it is apparent that these antibacterial properties have arisen as a result of evolutionary selection rather than as a result of exposure to specific microorganisms or antigenic determinants. Several non–antibody serum proteins can also be included among these.

Accumulation of metabolic intermediaries at sites of inflammation is associated with a lowering of the pH, which has an antibacterial effect, as have basic peptides and histones, which are released at the sites of inflammation by dead or dying cells.

Other Aspects of Nonspecific Immunity

Nonspecific immunity is controlled by genetic factors (as evidenced by interspecies differences in susceptibility and nonsusceptibility, racial and individual differences, and certain immunologic deficiency diseases, which are known to be genetically determined) and by the endocrine system (as evidenced by observations of susceptibility and nonsusceptibility influenced by hormones, adrenal hormones, thyroid hormones, sex hormones, and pineal hormones).

In addition, nonspecific immunity has been shown to be influenced by nutritional factors, age, and environmental and therapeutic factors such as exercise and exposure, irradiation, local wound care, drugs, and anesthesia.

Certain selected diseases have been shown to affect nonspecific immunity, either through an increase or decrease in the capacity for phagocytosis and/or a diminished or increased capacity for intracellular killing of bacteria. These include diabetes mellitus, cancer, and uremia. Prematurity and burn injury may also affect nonspecific immunity in this way, as can shock, infection, and alcohol intoxication.

Nonspecific immunity can be stimulated by drugs such as endotoxin (in small doses) tuberculin, zymosan, and restim. An increase in body temperature is also associated with an increase in metabolic function, which has a beneficial effect on nonspecific immunity. In addition, the transfusion of viable leukocytes from both normal donors and patients with chronic myelogenous leukemia is often effective in temporarily elevating the leukocyte count and thereby exerting a favorable effect on nonspecific immunity.

REVIEW QUESTIONS

MULTIPLE CHOICE

Choose the phrase, sentence, or symbol that completes the statement or answers the question. More than one answer may be correct in each case. Answers are given at the end of this book.

1. The process by which an animal resists the invasion of foreign or potentially harmful microorganisms by natural means (*i.e.*, without the production of protective antibodies) is known as:
   (a) humoral immunity
   (b) innate immunity
   (c) epithelial immunity
   (d) none of the above
   (Introduction)

2. The self-sterilizing power of the skin is achieved by:
   (a) desiccation
   (b) epithelial desquamation
   (c) pH
   (d) the secretion of fatty acids that have antibacterial properties
   (The Epithelial Barriers)

3. The mucosal surfaces of the body are protected by a so-called slime layer, which has been shown to possess antibodies of the:
   (a) IgM class
   (b) IgG glass
   (c) IgA class
   (d) IgE class
   (The Epithelial Barriers)

4. The classic signs of inflammation are:
   (a) pain
   (b) heat
   (c) redness
   (d) swelling
   (Inflammation)
5. The "slowing" of the blood stream is referred to as:
   (a) hyperemia
   (b) stasis
   (c) the vascular response
   (d) none of the above
   (Inflammation: The Vascular Response)
6. The fluid exudate in acute inflammation:
   (a) is formed as a result of decreased vascular permeability
   (b) has almost the same composition as plasma
   (c) contains drugs and antibiotics if these are present in the plasma
   (d) contains specific antibodies
   (Inflammation: The Vascular Response)
7. The cellular exudate in acute inflammation:
   (a) contains neutrophil polymorphonuclear leukocytes
   (b) has almost the same composition as plasma
   (c) enacts the process of phagocytosis
   (d) contains lymphocytes only
   (Inflammation: The Cellular Response)
8. The mechanisms contributing to phagocytosis include:
   (a) chemotaxis
   (b) opsonization
   (c) ingestion
   (d) degranulation
   (Phagocytosis)
9. An antibody that is present in a host and reacts with substances with which the host has had no known contact is referred to as:
   (a) innate antibody
   (b) humoral antibody
   (c) natural antibody
   (d) erythrocyte antibody
   (Nonspecific Humoral Immunity)
10. The biologic functions of complement in immune defense include:
    (a) cytolysis
    (b) immune adherence
    (c) chemotaxis
    (d) opsonization
    (Nonspecific Humoral Immunity: Complement)
11. Nonspecific immunity has been shown to be influenced by:
    (a) nutritional factors
    (b) age
    (c) environmental factors
    (d) therapeutic factors
    (Other Aspects of Nonspecific Immunity)
12. Nonspecific immunity may be stimulated by:
    (a) zymosan
    (b) anesthesia
    (c) leukocyte transfusions
    (d) all of the above
    (Other Aspects of Nonspecific Immunity)

ANSWER "TRUE" OR "FALSE"

13. The Duffy receptors on the red cell membrane are believed to be associated with susceptibility to tuberculosis.
    (Susceptibility and Nonsusceptibility)
14. The bacterial enzyme lysozyme is present in abundance in body secretions.
    (The Epithelial Barriers)
15. The antiviral agent known as *interferon* is synthesized by cells in response to viral infection.
    (The Epithelial Barriers)
16. In the early stages of the development of the cellular exudate in acute inflammation, the predominant cells present are monocytes.
    (Inflammation)
17. Leukocytes always display negative chemotaxis.
    (Chemotaxis)
18. Serum opsonins interact with the surface of bacteria, rendering it acceptable to the phagocyte.
    (Opsonization)
19. *Escherichia coli* is lysed by complement activation.
    (Complement)
20. Properdin exerts bactericidal and viricidal effects in the presence of the trimolecular complement complex C567.
    (Properdin)

General References

Alexander, J. W., and Good, R. A.: Fundamentals of Clinical Immunology. Philadelphia, W. B. Saunders Company, 1977.
Barrett, J. T.: Textbook of Immunology, 4th ed. St. Louis, The C. V. Mosby Co., 1983.
Bellanti, J. A.: Immunology II. Philadelphia, W. B. Saunders Company, 1978.
Bellanti, J. A.: Immunology: Basic Processes. Philadelphia, W. B. Saunders Company, 1979.
Henry, J. B. (Ed.): Clinical Diagnosis and Management by Laboratory Methods, 17th ed. Philadelphia, W. B. Saunders Company, 1984.
Parker, C. W.: Clinical Immunology, Philadelphia, W. B. Saunders Company, 1980.
Roitt, M.: Essential Immunology, 2nd ed. Oxford, Blackwell Scientific Publications, 1974.
Walter, J. B.: An Introduction to the Principles of Disease. Philadelphia, W. B. Saunders Company, 1977.

TWO

SPECIFIC IMMUNITY

OBJECTIVES

The student shall know, understand, and be prepared to explain:

1. The concepts of specific immunity
2. Antigens and antibodies
3. The structure of immunoglobulins
4. The general function of immunoglobulin
5. Immunoglobulin domains
6. Types of immunoglobulin, including:
   a. IgG
   b. IgM
   c. IgA
   d. IgE
   e. IgD
7. The function and characteristics of the above-mentioned immunoglobulins
8. Cells involved in specific immunity, including:
   a. The T-lymphocytes
   b. The B-lymphocytes
   c. Null cells
   d. NK cells

Unlike nonspecific immunity, the processes of specific (or "acquired") immunity are an adaptive response to foreign antigenic stimulus, which results in the acquisition of immunologic memory and the production of antibody, which reacts *specifically* with the antigen that caused its production.

Immunity against infectious diseases may be conferred basically in two ways: (1) by actual infections or inoculation that causes the production of specific protective antibodies (known as "active" immunity), or (2) by artificial transmission of antibodies, which afford temporary protection against invading antigen (known as "passive" immunity).

In some cases, a certain degree of "cross-immunity" is afforded when there is a relationship or similarity between causative agents. In this way, immunity against one infectious agent may contribute some immunity against other infectious agents.

Several factors are involved in the *degree* of protection produced as a response to infection or inoculation. In this connection, the size of the infecting dose, the route of administration, and the type of infective agent are contributory.

Active immunity may therefore be defined as "the state of resistance developed by an individual following effective contact with foreign microorganisms or their products." Such contact may be caused by:

1. Clinical or subclinical infection
2. The injection of live or killed microorganisms or their antigens
3. The absorption of bacterial products (*e.g.*, toxins).

The antibodies produced by the host in response to any of these events may take from a few days to a few weeks to develop, yet they usually persist for years, offering protection against reinfection. The agents used in active immunization are either

live, killed, or attenuated (*i.e.*, thinned: *L. attenuare* = to thin).

When foreign antigen gains entrance into the body, two types of immunologic reactions may occur. These are different but fundamentally similar mechanisms, known as humoral or cell-mediated immunity.

Humoral Immunity. Humoral immunity refers to the synthesis and release of free antibody (humoral antibody) into the blood and other body fluids. This antibody is then capable of direct combination with and neutralization of bacterial toxins by coating bacteria to enhance their phagocytosis, and so forth.

Cell-Mediated Immunity. Cell-mediated immunity refers to the production of "sensitized" lymphocytes, which take part in such reactions as the rejection of tissue transplants, the delayed hypersensitivity to tuberculin seen in persons immune to tubercle bacilli, destruction of cancer cells, and destruction of parasites.

ANTIGENS AND ANTIBODIES

As previously mentioned, active immunity against disease is achieved by the production of antibody (or cellular responses) in the host against a specific invading foreign antigen. An antigen is, in fact, any substance that is capable of stimulating the production of antibody. Antibody, conversely, refers to a group of plasma proteins (the immunoglobulins) that are formed as a result of exposure to antigen and that react *specifically* with that particular antigen. (Note: The outstanding characteristic of an antibody is its specificity; this implies that an antibody, once formed, is capable of reacting ONLY with the antigen that elicited its production.) Stimulation with a new antigen causes the production of a new antibody. This general rule is not absolute, as in cases of "cross-immunity"—see later in this text.

Antigens

An antigen is usually defined as any molecular structure that, when introduced parenterally into an animal, is capable of causing the production of antibodies by that animal. The antibody formed is capable of specific combination with the antigen that elicited its production. The antibodies produced are heterogenous with respect to immunoglobulin class, affinity for antigen, and specificity. At the present time, many authorities use the term *immunogen* when referring to antigen, and these terms have come to be used synonymously.

Antigenic substances of low molecular weight (*i.e.*, less than 5000) rarely stimulate the formation of antibodies and are known as *haptens*. These haptens can, however, provide antigenic specificity when coupled with a larger molecule. High molecular weight molecules of 500,000 or greater with complex protein or polypeptide-carbohydrate structures are the best antigens. The reason for this is that the entire molecule does not function as an immunoglobulin- or lymphokine-inducing structure. Instead, within each molecule, there are specific regions of limited size that function as the antigenic determinant sites, also known as *spitopes*. The number of these antigen determinant sites per molecule of antigen is referred to as the valence of the antigen. (Note: Valence, in this context, has no relationship to the ionic condition of the antigen.) It therefore follows that the larger a molecule is, the greater the number of antigenic sites, and thus the greater the variety and quantity of antibody that will be formed.

In addition to size, the foreign molecule must possess a chemical structure that is unfamiliar to the host. Again, the more diverse the chemical structure, the more antigenic the molecule becomes.

Lastly, the route of parenteral administration of the antigen is instrumental in the degree of antibody production. Generally, intravenous (into the vein) and intraperitoneal (into the peritoneal cavity) routes are effective. The intradermal (into the dermis, or skin) route offers stronger stimulus than the subcutaneous (beneath the skin) or intramuscular (into the muscle) route.

Several terms are used when describing antigens. These include autologous antigen, heterologous antigen, homologous antigen, and heterophil antigen. The meanings of these terms follow:

1. *Autologous antigen.* In simple terms, this refers to one's own antigen, which, under appropriate circumstances, would stimulate the production of autoantibody. Autologous antigen is therefore synonymous with autoantigen.
2. *Heterologous antigen.* This is merely a different antigen from that which was used in the immunization and which may or may not react with the antibody formed, depending upon the chemical similarity to the immunizing antigen.
3. *Homologous antigen.* This refers to the antigen used in the production of antibody.
4. *Heterophil antigen.* Also known as heterogenetic antigens, these are antigens that exist in unrelated plants or animals but which are either identical or so closely related that antibodies to one will cross-react with antibodies to the other.

Antibodies

Basically, antibodies may be viewed as substances produced in response to antigenic stimulation that are capable of specific interaction with the provok-

ing antigen. It has now become common practice when referring to antibodies to use the general term *immunoglobulin* because of the heterogeneity in the types of molecules that can function as antibodies. In humans, five distinct structural types or "classes" of immunoglobulin have been isolated: immunoglobulin G (abbreviated IgG), IgM, IgA, IgD, and IgE.

Structure of Immunoglobulins

Each of the immunoglobulin classes has a basic structural similarity. The single structural unit consists of two sizes of peptide chain, termed "heavy" and "light" chains. A model for the basic immunoglobulin molecule was proposed by Porter in the 1950s, which showed a symmetrical, four-peptide unit, consisting of two heavy chains and two light chains linked by interchain disulfide bonds (Fig. 2–1). The molecule appears to be Y-shaped, the arms of the Y swinging out to an angle of 180 degrees from the horizontal.

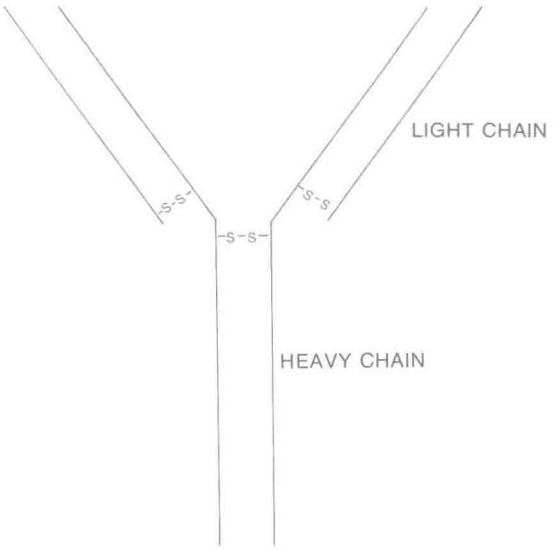

Figure 2–1. The model for the basic immunoglobulin molecule proposed by Porter.

In 1959, Porter demonstrated that treatment of IgG (which consists of a single structural unit) with the enzyme papain splits the molecule into three fragments. Two of these fragments appeared to be identical and were subsequently shown to be capable of binding specifically with antigen, although they were not capable of causing agglutination or precipitation reactions. These two fragments were called *Fab* (**f**ragment capable of **a**ntigen **b**inding); each is composed of one light chain and one half of one heavy chain. The remaining fragment (in rabbit IgG) was found to crystallize upon purification and was therefore called *Fc* (**f**ragment **c**rystalline). This Fc fragment was found to be composed of two halves of two heavy chains and was further found to be involved in complement activation, fixation to the skin, and placental transport, although it does not have the ability to combine with antigen (Fig. 2–2).

Treatment of IgG with the enzyme pepsin results in a slightly different Fab-type fragment that not only retains the ability to bind with antigen but is also capable of causing agglutination or precipitation reactions. This fragment is known as $F(ab')_2$. It has two antigen-binding sites and is composed of two light chains and two halves of heavy chains. The remainder of the molecule is split into many small fragments by pepsin digestion (Fig. 2–3).

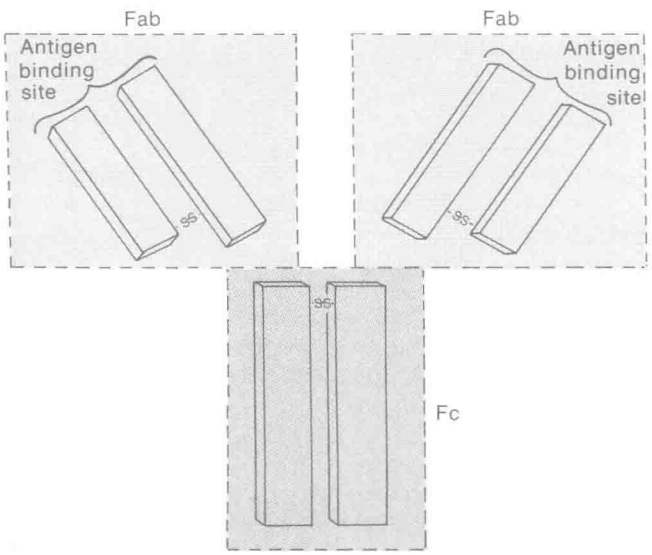

Figure 2–2. Cleavage of antibody molecule by papain.

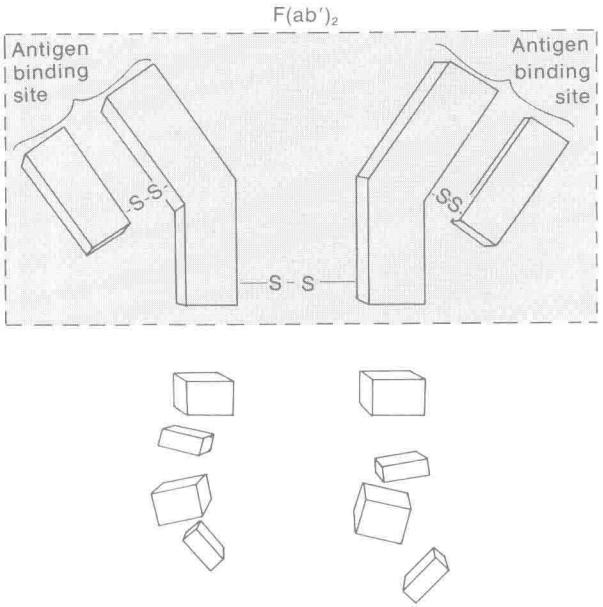

Figure 2–3. Cleavage of antibody molecule by pepsin.

These findings provided the following information: (1) The Fc portion of the molecule directs the *biologic activity* of the antibody molecule (*e.g.*, placental transfer of IgG and complement fixing). (2) the Fab portion is involved in antigen binding.

Further studies of the peptide chains have been made by analysis of enzyme digestion products of myeloma proteins. (In the disease known as multiple myeloma, a cell making a particular immunoglobulin repeatedly divides in an uncontrolled way. The patient then possesses enormous numbers of identical cells derived as a clone from the original cell, all synthesizing the same immunoglobulin—the myeloma protein, or M-protein, which appears in the serum, often in high concentrations. Purification of the myeloma protein renders a preparation of an immunoglobulin with a unique structure.)

Analysis of a number of purified myeloma proteins has revealed that the amino acid sequence of the heavy and light chains contains a "constant" region, where the amino acid sequence is identical for the type and subtype, and a "variable" region, consisting of the first 110 to 120 amino acids, which vary widely between type and subtype. The variable part of the peptide chains provides specificity for binding antigen; the constant part is associated with different biologic properties, which vary from one immunoglobulin class to another (Fig. 2–4). In addition, studies of the light chains using the Bence Jones protein (found in a proportion of patients with multiple myeloma) revealed that they could be divided into two groups, called kappa (κ) and lambda (λ). Whereas each immunoglobulin class is associated with a particular type of heavy chain, each myeloma protein studied thus far, whatever its class, has possessed light chains of either kappa or lambda specificity, but never of both together.

General Function of Immunoglobulin

For each molecule of antigen, millions of specific antibody molecules may be produced and secreted into the body fluids. Basically, the function of antibody is to neutralize toxic substances, to facilitate phagocytosis and kill microbes, and to combine with antigens on cellular surfaces and thereby cause the destruction of these cells either extravascularly (outside the blood vessels within the re-

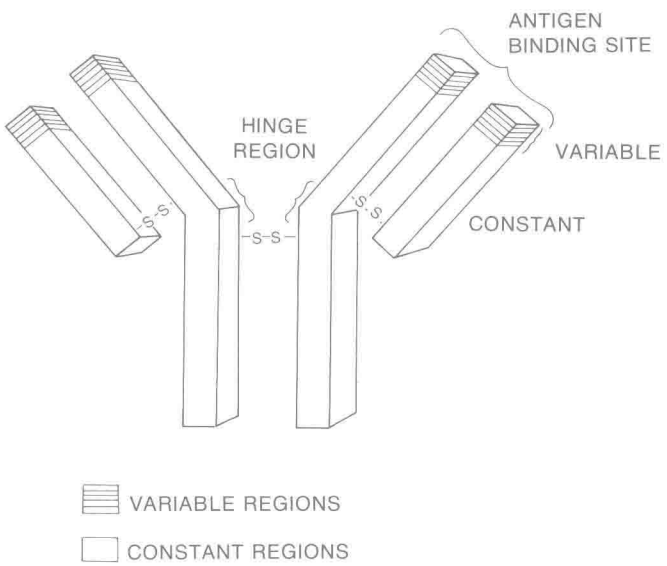

Figure 2–4. IgG Molecule, showing constant and variable regions.