TEXTBOOK OF IMMUNOLOGY

Second Edition

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Preface to the Second Edition

We have made a series of changes in order to accommodate the mass of new information that became available during the last four years. As before, this book has been designed as an introductory textbook with heavy emphasis on basic immunological phenomena. The major revisions concern the new information on immunoglobulin genes, the analysis of histocompatibility and the function of T cells. The latter two, probably the most difficult topics for the students, are presented, gradually, in four chapters. First, we present a general description of the histocompatibility molecules and their genes (in Chapter 6), followed by their function in transplantation and physiological cellular interactions (in Chapters 6, 7, and 8). Lastly, in Chapter 10, we describe the function of immune response genes as originally discovered.

We are grateful to our many colleagues who helped us with their advice and comments. To note is that Dr. Kurt J. Bloch revised all of Chapter 3, and, with Dr. Susan Canelosi, a great part of Chapter 4. Dr. K. F. Austen revised the complement chapter. Dr. F. S. Rosen revised Chapter 15. Dr. Ronald N. Germain wrote parts of Chapter 11. We also acknowledge the help, in this second edition, of Drs. Martin E. Dorf, Edmond J. Yunis, and Elvin Kabat. Both George F. Schreiner and F. S. Rosen read most of the chapters and provided considerable help on editorial and scientific matters. We are grateful to Pamela Battaglino, who drew all the new graphs of this edition. Finally, we owe a great deal to Barbara K. Gricus, who typed the whole book and also helped us with editorial matters.

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Boston, Massachusetts
November, 1983

Preface to the First Edition

The knowledge of immunology has become essential to students of medicine and biology. The applications of its methodology to many fields in the biological sciences are widely recognized and extensively utilized. Furthermore, immunology has been highly successful in applying a variety of fundamental technologies derived from biochemistry, genetics, cell biology, and pathology to the analysis of highly complex phenomena which constitute the immune response. The study of modern immunology has provided us with critical information applicable to other fields of biology and medicine. The differentiation of the lymphoid system and the functions of its component lymphocytes offer to the cell biologists the best analysis of complex cellular systems. The intricate interactions among these cells and the genetics which govern these phenomena are models for other differentiating systems. Furthermore, the genetic problems presented by the generation of diversity in immunoglobulin-combining sites have important applications to molecular biology and genetics. Likewise, the study of complement and anaphylaxis has provided information essential for our understanding of inflammation and tissue pathology.

This textbook is designed to provide information which will permit a medical student, a college student, or a graduate student in the biological sciences to have a basic understanding of the rapidly expanding and complex discipline of immunology. The various chapters cover the entire field describing the basic immunological phenomena, the biochemistry of antibodies and antigens, the immunological techniques, the cellular and genetic components of the immune response, and, finally, immunopathology. The material presented is sufficiently complete to permit an in-depth understanding of immunological phenomena, as well as to serve as an introduction to more extensive studies.

This textbook was originally organized from teaching materials prepared for first-year medical students at Harvard Medical School and provided to them as part of the introductory course in immunology of the Harvard Medical School curriculum. The chapters follow the same sequence as the lectures in the course and cover the same material.

Several chapters were written by colleagues at Harvard Medical School. Dr. Kurt J. Bloch wrote the chapters on antibody structure and with Dr. Susan Canelosi wrote the method chapter; Dr. K. Frank Austen wrote the chapter on complement; Dr. Fred S. Rosen wrote the chapter on immunodeficiency; and Dr. Ronald N. Germain wrote the chapter on tumor immunity. Other colleages helped us by editing certain sections or providing us with their advice and opinion. For this we thank Drs. Kenneth A. Ault, Martin E. Dorf, Eric Martz, and Edmond J. Yunis. Lastly, we thank the first-year Harvard Medical students who, by giving us their criticisms, involuntarily, have taught us how to teach immunology.

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September, 1979

Contents

CHAPTER 1

Immunology: Principles and Phenomenology

This chapter is an overview of some of the most important immunological phenomena placed in a historical context. This overview is needed as an initial step preceding the detailed analysis of immune cells and molecules and their interactions.

Early History of Immunology: The roots of immunological phenomena are very old. They reside in ancient observations, recorded in disparate cultures, that patients who survived infectious diseases seldom contracted that disease again. Immunology as a science did not emerge until relatively recent. Indeed, one can date it to eighteenth-century England, a time of recurrent epidemics of smallpox. Smallpox was a serious viral disease characterized by infective lesions in internal organs and skin. In the skin, the lesions consists of fluid-containing blebs in the epidermis that break, ulcerate, and scar. At that time, one way to avoid smallpox was through the practice of "variolation", which had its origins in the Middle East. The infective fluid from the pox lesions was inoculated into an individual for the purpose of producing a limited infection. Such individuals were then protected from acquiring the severe disease during the epidemics. Variolation had a limited success since it was difficult to control the extent of infectivity, and frequently, a severe disease would develop. We owe to Edward Jenner the first attempt to "immunize" deliberately against an infectious disease, using an attenuated non-infectious form of the microorganism. At the time that these studies were done, however, there was no knowledge of microbes as the cause of infection. When Jenner was an assistant in practice, delivering "primary care", a young country woman told him that she could not have smallpox since she had already contracted cowpox. Cowpox was a pox virus disease, endemic in cattle, frequently observed in milkmaids. The viruses of cowpox and smallpox share antigens. Jenner first inoculated James Phipps with cowpox infectious crusts and, later in time, practiced variolation on him with small amounts of smallpox infective material. Phipps had developed immunity to the smallpox by the inoculation with cowpox, and no infective lesions developed. Thus, Jenner's attempts were successful, and he concluded that the mild infection with cowpox protected against the severe infection with smallpox. He published his classical

memoir: "An inquiry into the causes and effects of the variolae vacciniae" in 1798. Less than two centuries later, the World Health Organization would declare smallpox extinct as a disease, as a result of a worldwide vaccination program.

Our understanding of Jenner's experiment did not come until years later with the formulation of the germ theory of disease, based to a major extent on the experimental work of Louis Pasteur. Although initially interested in bacteria and their pathogenic properties, Pasteur became increasingly concerned with the prevention of infectious diseases and, thereby, became the first great experimental immunologist. Even though comparatively little was understood of the ways in which various bacterial pathogens caused disease, Pasteur realized that methods for immunization could be developed. These were initially based upon observations made with the chicken cholera bacillus. A level of immunity comparable to that elicited by the virulent organisms in surviving subjects could be stimulated by "attenuated" or modified strains. These strains were able to stimulate immunity without causing disease. This experimental analysis permitted the original success of Jenner's vaccination experiments to be understood. Pasteur, in honor of Jenner, called his treatment "vaccination". Vaccination is the method for stimulating immunity to an infectious disease by injection of the microorganism or its products.

It is easily understood, therefore, that immunology initially grew as a subdiscipline of bacteriology and was concerned primarily with the study of the development of immunity and specific defense mechanisms against infectious agents and their toxic products. The discovery of antibodies was the major accomplishment of this phase of immunological research. Roux and Yersin in 1888, at the Pasteur Institute, found the bacterial toxin of the diphtheria bacillus in the culture filtrates. Later immunity against the toxin was ascribed to the development of specific neutralizing substances or antitoxins in the blood of the immune animal, giving rise to the concept of antibodies. Such antitoxin could be passively transferred to a non-immune animal with immune serum. This result was first observed in the case of tetanus antitoxin by Von Boehring and Kitasato in 1890 at the Koch Institute in Berlin. The specific substances found in the serum of immune animals able to confer passive immunity were called antibodies and were the subject of intensive scrutiny. Antibodies were able to:

1. neutralize toxins

2. agglutinate bacteria

3. kill and lyse bacteria in the presence of normal serum. The components in normal serum required for lysis were found to be heat labile. Initially called alexine and later complement by Bordet, they are now known to include sixteen interacting serum proteins (Chapter 12).

4. opsonize bacteria for phagocytosis by macrophages or polymorphonuclear phagocytes

5. sensitize passively for anaphylactic (allergic) reactions.

All these phenomena displayed a high degree of specificity for the antigen that induced the antibody. They were generally protective for the patient who developed this immunity and constituted fundamental defense mechanisms against infectious agents. The immune sera which display these characteristic properties could confer passive immunity to normal, non-immune recipients. This form of immunotherapy, still in use today, is termed serotherapy.

The discovery that the antibody properties of antisera are attributable to specific protein molecules was made by Michael Heidelberger who showed that pneumococcal capsular polysaccharides precipitated specific serum proteins from rabbit anti-pneumococcal antisera. These antibodies or immunoglobulins are the molecules responsible for the precipitating, agglutinating, and complement-fixing properties of specific antisera. It was later established by A. Tiselius and E. Kabat that the bulk of antibodies in hyperimmune antisera had the electrophoretic properties of gamma globulins. The subsequent concern of immunologists was, for many decades, centered upon the properties of antibodies, their structure, the stimulation of their synthesis, and the nature of the complement system.

ANTIBODIES AND ANTIGENS

Antibodies are molecules synthesized upon stimulation by antigen and endowed with exquisite specificity for the antigen. Antigens are molecules which, when introduced to a subject, are able to stimulate the synthesis of specific antibodies capable of interacting with them. The structure of antibodies and the various classes to which they belong will be discussed in Chapter 3. At this time, we simply need to state that antibodies react with antigens by virtue of their antigen-combining sites and that each antibody molecule is made up of two identical half molecules, each containing a heavy and a light chain. Each molecule binds to one antigenic determinant at one of its ends where both heavy and light chains associate to create the combining site. The antigenic determinant is a small portion of the antigen, no more than a few amino acids long. Thus, antibody molecules bind two identical antigenic determinants. Interaction of antibody and antigen depends upon the steric configuration of both molecules, permitting a close fit between the antibody-combining sites and the corresponding determinant of the antigen.

The antibody molecules, besides binding to antigen, can interact with inflammatory cells and soluble molecules and thus can modulate important biological phenomena—such as cell lysis, phagocytosis, degranulation, and secretion of intracellular components from neutrophils, macrophages, and mast cells, etc. Antibody molecules are grouped into five major classes (IgG, IgM, IgD, IgA, and IgE) on the basis of structural differences in heavy chains. Each Ig class modulates specific biological effects, although each can bind to the same antigen with the same high degree of specificity. Thus, the antibody molecule is unique in that it is endowed with two distinct properties: the specific one of binding to unique antigens and the non-specific one of interacting with inflammatory cells and/or soluble serum components.

An important concept to bear in mind is that practically all natural antigens possess several antigenic determinants, each capable of eliciting antibodies. In addition to the heterogeneity in the antibody response to an antigen, resulting from numerous determinants on an antigen, the population of antibodies produced against a single determinant is also highly heterogeneous with respect not only to class of Ig but also to binding affinity, a reflection of the primary amino acid sequence of the combining site of the molecule (Chapter 3). The antibody register is, therefore, of enormous size and possesses a wide scope as well as marked specificity. Thus, we must consider antibodies produced against a given antigen as a population of antibodies with varying degrees of affinity for the various determinants of the antigen. As one would expect, therefore, antibodies produced against molecules with related structures will crossreact to a considerable extent, although a small population within these antibodies can be shown by absorption to be absolutely specific for the immunizing antigen. The degree of crossreactivity depends upon the structural similarities of the antigens. Thus, bovine serum albumin (BSA) and human serum albumin (HSA) stimulate the synthesis of rabbit antibodies which are extensively crossreactive, whereas anti-human serum albumin and anti-human gamma globulin (HGG) antibodies do not crossreact.

The study of the specificity of antibodies has depended very much upon the understanding of antigenic determinants. We owe to Karl Landsteiner the discovery that small, defined chemicals such as 2,4-dinitrofluorobenzene (DNFB) could be covalently bound to immunogenic molecules to form new antigens, the response to which is directed against the chemical group introduced. Such a chemical group which by itself is too small to stimulate a response but, nevertheless, reacts with the antibody, has been called a hapten. (The immunogenic protein to which the hapten is bound is termed the carrier.) Thus, antibodies against DNP-BSA will react with DNP-HGG. In solution, this interaction of soluble DNP-HGG with the antibody at an appropriate ratio results in the formation of a lattice of crosslinked molecules, which, as it grows, becomes insoluble and precipitates (Chapter 4). This reaction is inhibited by a monovalent hapten such as DNP bound to a small chain of lysines (Chapter 2).

What are the properties of antigens essential for immunogenicity? Chapter 2 analyzes this question. We can state now that the following classes of molecules are generally antigenic: 1) foreign proteins and polypeptides of size larger than eight amino acids; and 2) polysaccharides with repeated structural units. Generally speaking, small chemicals, lipids, and nucleic acids are weakly immunogenic. They can, when conjugated to an immunogenic protein carrier, behave as haptens and stimulate specific antibodies against these determinants.

The dissociation between antibody specificity and immunogenicity suggests the existence of separate recognition systems for the hapten and the carrier molecule at the cellular level in the induction of antibody responses (Chapter 8). The degree of immunogenicity of a molecule depends fundamentally on the extent to which such a molecule differs from homologous components of the host, since, generally speaking, autologous components which are strong antigens in a foreign species, are not immunogenic in the host of origin under normal conditions.

PRIMARY AND SECONDARY ANTIBODY RESPONSES

When a conventional antigen which an individual has never encountered is administered for the first time, one observes a latent period lasting several days during which antibodies are not detected in the serum nor antibody-producing cells in the lymphoid tissues. The more sensitive the method, the shorter is the latent period, but it is rarely less than three or four days. Following this period, the amount of antibodies in the serum increases exponentially to reach a peak level and then decreases. This is the primary response. It usually consists of IgM antibodies with sometimes a small IgG component appearing later, depending upon the antigen, the dose, and the route of immunization. If the animal is left to rest until the serum antibody concentration declines to low or undetected levels and then a similar or lower amount of the same antigen is administered, the second antibody response is much more rapid and stronger, the latent period is shorter, and the antibody produced is predominantly IgG in character. After reaching its peak, the secondary or anamnestic response decreases much more slowly. This is the phenomenon of immunological memory on which all prophylactic immunizations are based (Figure 1.1).

Another important feature of the antibody response is the increase of the average affinity of the antibody for the antigen with time of the immunization. This is generally referred to as maturation of the immune response. Increased antibody affinity with time has been observed both with conventional antigens such as diphtheria toxin,

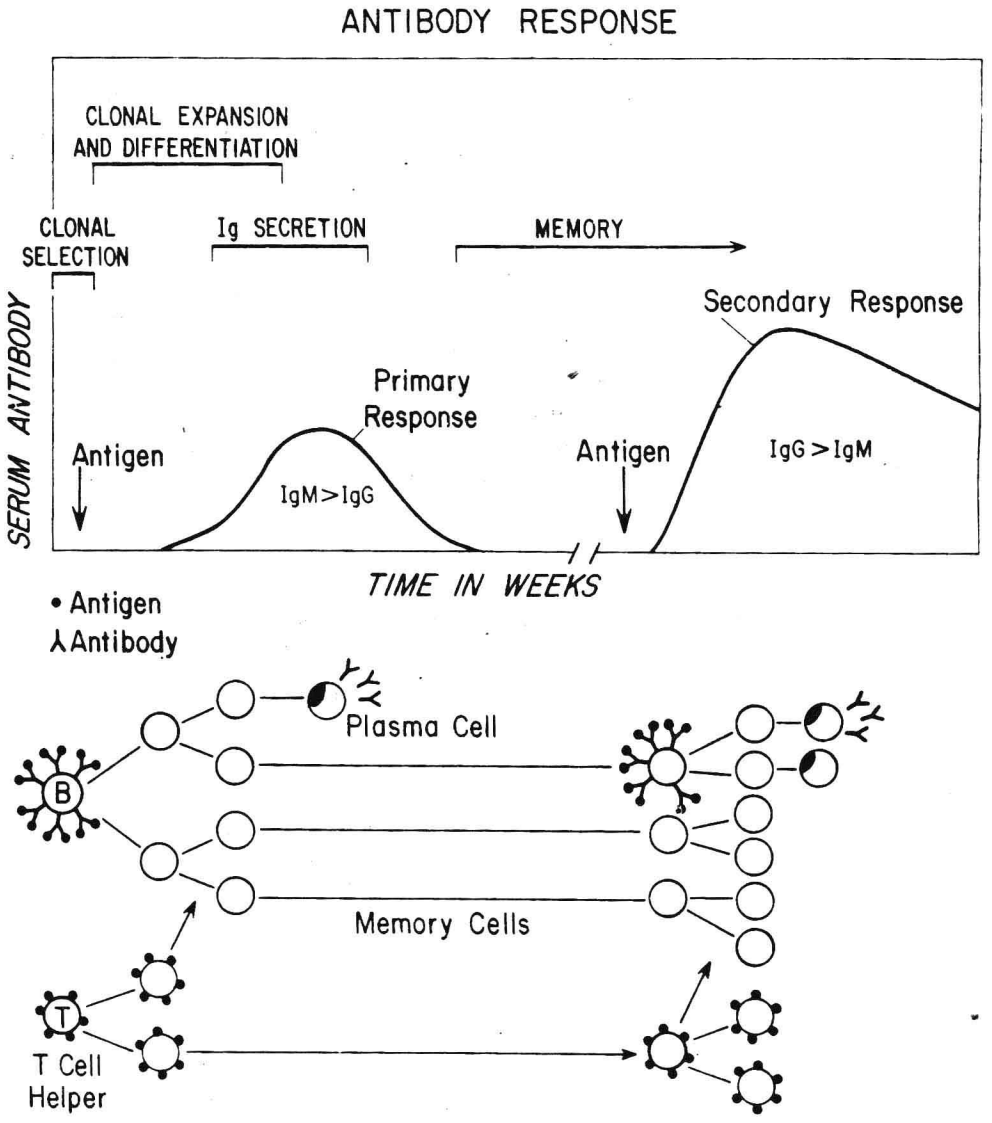

Figure 1.1. Antibody response in the serum against a conventional thymus-dependent antigen. The top panel depicts the serum levels of antibody after primary and secondary challenges. The bottom panel illustrates the cellular events in B cells, T cells, and plasma cells.

influenza virus, and hapten-protein conjugates. The studies with anti-hapten antibodies of Gregory Siskind and Herman Eisen have provided precise affinity measurement for this phenomenon. Thus, in the course of immunization, the affinity of late antibodies can increase by 10,000-fold over the affinity of early antibodies. In addition, this phenomenon is very much dependent upon the dose of antigen administered: the rate of maturation of antibody affinity increases with decreasing doses of antigen until a maximum is reached; if too little antigen is administered, antibody affinity no longer increases optimally.

The phenomenon of increase in antibody affinity with time of immunization is extremely important. The efficacy of humoral immunity depends as much, if not more, upon the affinity of the antibody for the antigen than upon the amount of antibody produced. Indeed, most defense phenomena involving antibody occur in body fluids at very low concentrations of antibodies and antigens.

THE CELLS OF THE IMMUNE RESPONSE

The events in the serum reflect the cellular dynamics of the immune response. The immune response results from the interaction of the antigen with lymphocytes bearing specific receptor proteins on the surface membranes. Lymphocytes are very heterogeneous cells that are grouped into two major classes, the T cells and the B cells. The T cells originate from the thymus gland where they mature and differentiate following interactions with the thymic stromal cells. The mature T cells then leave the thymus gland to continuously circulate from blood to lymph nodes and back to blood. T cells are represented by three major subsets: the T helper cells, the T suppressor cells, and the T cytolytic cells. T helper cells, as their name implies, regulate—help—other cells: B cells to secrete antibody, T cytolytic cells to become functional, and macrophages to become highly activated. Many of these functions are carried out by proteins elaborated by the T cell, called lymphokines or lymphocyte mediators (Chapter 7). The T suppressor cell functions to limit and terminate the immune response (Chapters 8 and 9). Immunity thus represents a fine balance between helper and suppressor functions. Shifts in this fine balance may result in abnormalities of immune function. T cytolytic cells are cells that attach to and kill tumor cells or virally infected cells (Chapters 7 and 11).

The B cells, or antibody-forming cells, derive from immature precursors found in abundance in bone marrow. When mature, the B cells are found in lymphoid organs in clusters called lymphoid follicles (Chapter 5). B cells interact with antigen by way of antibody molecules bound to their plasma membranes, acting as receptor proteins.

Following interaction with antigen and T cells, the B cells differentiate to antibody-forming cells called plasma cells. Plasma cells secrete antibody with the same specificity for antigen as that found in the receptor of the B cell from which it derived. This basic function of B cells, to have antibody as receptors and to secrete antibodies when stimulated, were hypothesized in 1902 by Paul Ehrlich at a time when there was no knowledge of lymphocytes nor of cell receptors. Ehrlich side chain theory stated that cells making anti-toxins had protoplasmic extrusions that would bind to the toxin and that this binding would induce more side chains that would then be shed to the outside, appearing as serum anti-toxins. Ehrlich's theory came too early in time to be understood, and no cellular work of significance was done until very recently.

By early 1950, theories explaining the great diversity in the specificity of antibodies fell into two groups: the instructive and the selective. The instructive theories postulated that antigen acted as a template over which antibody molecules folded and acquired their correct complementary configuration. The instructive theories were abandoned when it became evident that antibodies of different specificities had different amino acid sequences in their combining sites. The selective theories were first proposed by Niels Jerne and David Talmadge and postulated that antibodies of all specificities are produced prior to antigen administration, at low levels, and that the antibody molecule itself acts as the selective device to bind antigen and to permit specific antibody synthesis to be stimulated. Our understanding of immunologic phenomena was greatly fostered by the crucial contribution of Sir McFarlane Burnet, who envisaged selection at the level of the cell and proposed the "clonal selection theory of immunity". According to Burnet and the clonal selection theory:

1. Immunocompetent lymphocytes bear antibody receptors on their cell membrane. These receptors have identical specificity for antigen as the antibodies synthesized by their progeny when differentiated and activated.

2. Each immunocompetent lymphocyte bears antibody receptors of unique specificity (Figure 1.2).

We know now that each B cell reacts with a unique antigenic determinant, i.e., all the receptors on a B cell are monospecific. Thus, the immune system, as originally postulated by Burnet, functions by antigen binding to those lymphocytes with unique receptors of high affinity.

These selected lymphocytes are committed to bind antigen during their differentiation, prior to contact with antigen. The binding of antigen stimulates the selected B cells to divide, producing daughter cells of the same specificity. Clonal selection explains the progressive increase in antibody affinity with time. There is an

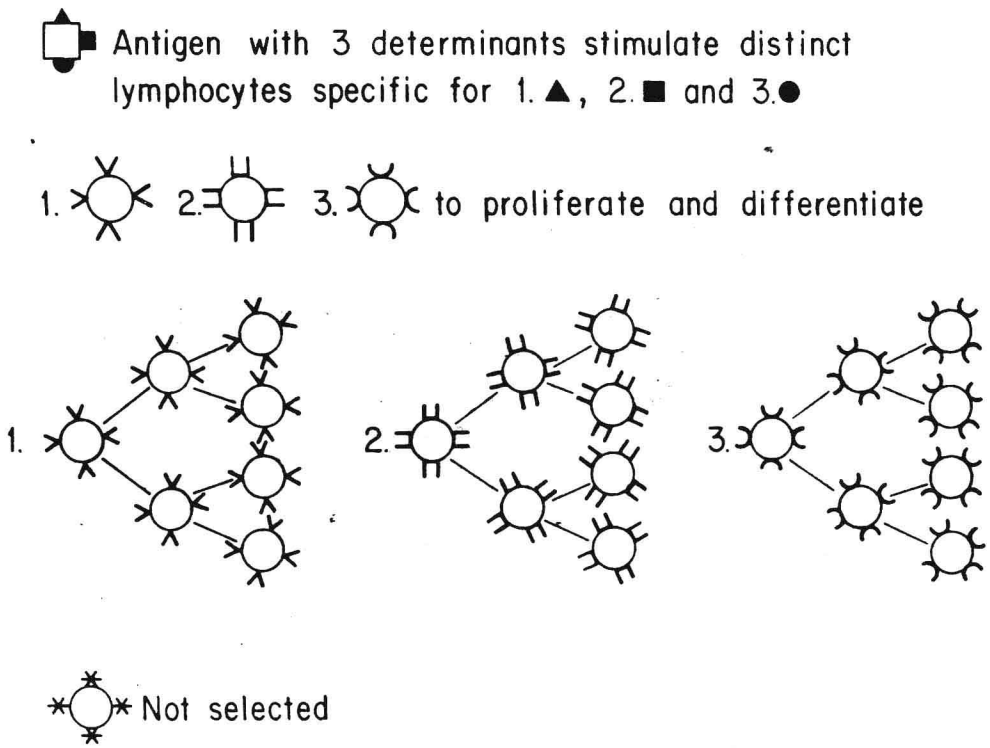

Figure 1.2. The figure depicts Burnet's Clonal Selection Theory. Different determinants on an antigen stimulate the response of different lymphocyte clones, each bearing antibody receptors for one of the determinants.

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antigen-driven shift in favor of B cells producing antibodies of higher affinity. As antigen is metabolized and its concentration decreases, the high-affinity B cells will be preferentially stimulated to proliferate and differentiate.

The interaction of T helper cells with antigen, which is a fundamental step in the immune process, is highly complex, involving interaction with antigen-handling cells like the mononuclear phagocyte that serve as antigen-presenting cells (Chapters 5, 6, 7, and 8). This interaction involves the recognition by the T cell not only of antigen

(by receptors not yet characterized biochemically) but of surface molecules of the phagocyte encoded in the transplantation gene locus of the species (Chapter 7). These histocompatibility molecules were initially recognized when studying the phenomenon of graft rejection. Relatively recently, it became clear that histocompatibility molecules serve to regulate the key interactions between helper T cells and phagocytes—and also between T cytolytic cells and their target cells. The histocompatibility locus codes for highly polymorphic surface glycoproteins grouped into two major classes—I and II, distinct in their chemical structure and biological effects. Curiously then, the recognition of a foreign molecule by the helper T cell is also dependent upon the recognition of the glycoproteins of the histocompatibility complex. This restriction in recognition may be a device to limit and control the response.

The immune system will not recognize self-antigens not because of the lack of development of autoreactive B and T cells but due to regulatory interactions taking place during fetal life. These interactions will induce a state of unresponsiveness or tolerance to the autologous antigenic determinants (Chapter 9). The presence or absence of immune response to a polypeptide antigen depends upon an effective interaction during immune recognition among the antigen, the T cell receptor, and the histocompatibility gene products of the presenting cell.

EFFECTOR FUNCTIONS

In order to carry out its function of protective immunity, the immunological system has developed a widespread number of effector reactions that result from the combination of antibodies with antigen and by the activation of T cells. As mentioned before, antibody molecules belong to five different subclasses. The IgM and IgG molecules are highly effective in fixing and activating complement thus generating a number of molecules that produce acute inflammation (Chapter 12). The IgG molecule, particularly when complexed to antibody and complement, binds avidly to surface structures of neutrophils and macrophages, enabling the complex to be phagocytized at a very high rate. IgG molecules cross the placenta and offer protective immunity to the fetus and the newborn. IgA molecules are found in secretion and serve in the elimination of some bacteria and viruses from mucosal surfaces. IgE molecules are found in trace amounts in blood but bind with very high affinity to mast cells. When antigen binds to the bound IgE, the mast cell degranulates, releasing its potent vasoactive amines that cause vasodilation and increase in permeability. IgE molecules were discovered for their harmful effect—the allergic response is a manifestation of an abnormal IgE antibody response (Chapter 14).