THE CELLULAR BASIS OF THE IMMUNE RESPONSE

An Approach to Immunobiology

Edward S. Golub

PREFACE

This book has grown out of a course in immunobiology which I have taught at Purdue University for the past several years. The course attempts to present an up-to-date overview of the biology of the immune response. It is not a comprehensive course covering all aspects of immunology. The emphasis is on cell interactions and regulation, subjects which are in my own specialty, but it also covers a good amount of immunoglobulin structure and the generation of diversity. In teaching the course, I try to illustrate as many points as possible with experimental design, as well as to describe the rationale and, when possible, the historical sequence of the experiments. I've tried to do that as much as possible in this book as well as to catch the flavor of how science is done and convey it in as pleasant a manner as I can. In choosing the particular experiments to illustrate the points which I have decided to make, I will no doubt shock, anger, or insult many friends and colleagues. I apologize to them for my idiosyncratic views but hope that by the time they finish reading the text they will become converts.

I have attempted to keep names of individuals to a minimum in the text. Inclusion of many names works a hardship on students, so I have included only the names of a few individuals and apologize to my colleagues (and I hope still my friends) who were not mentioned. Please know that I thought of each of you as I left your name out.

It must be emphasized that this book is not intended to be a compendium of facts. It is intended as a sweeping overview of the experimental basis of modern cellular immunobiology. Practicing cellular immunologists will be familiar with everything which is covered. The book is intended for upper-division undergraduates, graduate students, medical students, and scientists working in other areas of biomedical research who wish to redeem their lost youth.

If the book is used as a text in a formal course, I highly recommend sending the students to the literature to read original papers since I could not give critical evaluation of data in this book.

Finally I thank the many friends and colleagues who read parts of the manuscript and made so many helpful suggestions or who were so helpful in discussions of interpretations of data and trends and in

sharing unpublished data. Their names are listed below. Any shortcomings in the text are to be blamed on them. I take full credit for any value in this book.

Frank Adler, Bernard Amos, Dick Asofsky, Dick van Bekkum, Harvey Cantor, Max Cooper, Gus Cudkowicz, Tony Davies, Karel Dicke, Jeaninne Durdik, Dick Dutton, Ger van den Engh, Marc Feldmann, Dick Gershon, Joel Goodman, Mel Greaves, Howard Grey, George Jannosy, David Katz, Elaine McDaniel, Rick Miller, Av Mitchison, Andres Mulder, Peter Panfili, Ben Pernis, Jim Prahl, Martin Raff, Janet Roman, Larry Ruben, Jim Russell, Stu Schlossman, Eli Sercarz, Liz Simpson, Greg Siskind, George Snell, Osias Stutman, Jim Till, Dennie Toth, Mary Ann Wagner, Leon Wofsy, Ed Yunis, and Rolf Zinkernagel. My thanks also to the students of Biol. 537 at Purdue, Biol. 185 at UCLA, and Biochem. C55 at Northwestern who gave, often with brutal frankness, a student's view of the book. Special thanks to Patrick Nickoletti, Marge Ramirez, and Fran Selleck for suffering through the typing of the manuscript, to the staff at the Jackson Laboratory, Bar Harbor, Maine, for hospitality while I was writing, and to Andy Sinauer for holding my hand through the trauma of doing the book.

Edward S. Golub
Lafayette, Indiana
November, 1976

CONTENTS

1

INTRODUCTION AND THE NATURE OF SELECTION IN THE IMMUNE RESPONSE

OVERVIEW

The study of immunology has its historical roots in clinical medicine. The word itself derives from the Latin *immunitas* which means freedom from a public service and later came to be used to indicate freedom from disease. The ancients realized that after exposure to disease or recovery from a disease an individual was less susceptible to that disease. The Chinese practiced a form of vaccination long before Jenner, and Jenner himself was able to vaccinate against smallpox not because of exact technical knowledge, but rather from the observation that milkmaids, who had scars of the pox on their hands from cow pox (an occupational hazard), had fewer scars on their faces from smallpox than the rest of the population.[1] The history of immunology is really the story of the elucidation of the mechanisms behind this freedom from disease through prior contact.

Ironically, this book will not deal very much with either disease or the freedom from disease. Immunologists have found in the last few decades that the study of model systems which are not harmful yield a more fruitful means of studying the mechanisms of immunity. We should emphasize right at the outset that the mechanisms which the body uses to react to harmful substances are the same that it uses in reacting against nonharmful substances. Indeed, this unity of

[1]The Dutch scientist-musician G. J. van den Engh has pointed out to me the likelihood that milkmaids were generally known to be something special. The number of milkmaids who are chased through fields singing "fa la la" in English folk tunes far exceeds that of scullery maids, nannies, or seamstresses.

mechanisms has made immunology a useful and attractive subject, since fundamental mechanisms about disease processes can be studied in the absence of disease. So in this book we will be talking about the response of animals, usually the mouse, to such distinctly nonpathogenic substances as sheep red blood cells, bovine serum albumin, and keyhole limpet hemocyanin. The characteristic that all these diverse substances have in common is that they are foreign to the animal they are injected into, and this is the key to the immune response. *The body recognizes substances that are foreign and makes specific responses against them.*

THE NATURE OF SPECIFICITY

We will see in the course of this text that the immune response can be loosely divided into two types, antibody formation and cell-mediated responses. All the phenomena we will be examining are the result of events occurring in and on cells. The predominant cell type in the immune response is the lymphocyte. The aspects of the immune response involved in antibody formation are generally referred to as HUMORAL and those involved in tissue reactions as CELLULAR. It will appear in some parts of this text that they are really separate and distinct kinds of phenomena, but this is not the case. To study something in science, one must isolate it from as many other factors as possible, and in doing this, the illusion of separateness is often maintained after the experiment is over. Because of this, one must always try to fit the isolated observation, or more accurately, the observation of the isolated, into a total picture. In the case of the immune response, the humoral and cellular aspects are really parts of a continuum of responses, and the cells and events involved in one may overlap the other. To study either, however, attention is focused on the particular one being studied.

The humoral aspect of the response refers to the producing of serum antibodies. When a foreign material—called an ANTIGEN—is injected into an animal, a complex series of events occurs. One result of these events is the appearance in the serum of molecules which can specifically combine with the antigen. These molecules are called ANTIBODIES. The antibody molecule is one of the best studied of protein molecules, and its structure and nature will be covered in detail in Section III. At this time it is important to emphasize only that in response to a specific antigen the animal responds with the production of proteins which can combine specifically with the antigen. Thus, if a mouse is injected with sheep red blood cells, after an interval there will appear in the serum antibodies which combine

with the sheep red blood cells. These anti-sheep red blood cell antibodies will not react with horse red blood cells or bovine serum albumin. Now the very interesting point is that the molecules which have this specificity are of a type (gamma globulins) which are present in the serum at all times. It is not the production of a new *kind* of molecule that has been induced but rather of new combining *specificities* within the globulins. The specificity of these molecules will be shown to be due to differences in the sequence of amino acids in only a very small portion of the peptide chains making up the antibody molecule. Each specificity is coded for in the DNA of the cell producing the antibody since it is part of the dogma of modern biology that the order of amino acids in a peptide reflects the order of the nucleotides in the DNA of the cell which produced the peptide.

The antibodies mentioned above are produced by cells called LYMPHOCYTES. Thus the study of the humoral part of the immune response is also the study of the cellular basis for antibody production since antibodies are produced by cells. But the lymphocytes can be subdivided into at least two classes or types called B-CELLS and T-CELLS. One of these classes of lymphocytes, the B-cell, is responsible for the synthesis and secretion of antibody molecules. The other, the T-cell, is responsible for helping the B-cell do this, but T-cells are also able to carry out a whole series of reactions on their own. Generally these are reactions which involve tissue destruction. Since antibody is not involved in these reactions, they are called CELL-MEDIATED REACTIONS. Such reactions have the same (or very similar) ranges of specificity as do humoral reactions. That is to say, the tissue of an animal has chemical groupings which are antigenic in some other species and can therefore be recognized as foreign and reacted against. Instead of a reaction leading to antibody formation, however, the responding cells may react directly with the antigen on the foreign tissues and cause destruction of that tissue. Some current thinking about the body's defense against tumors is that a tumor has unique antigens and is therefore recognized as foreign. The immune system then responds against the new antigens, and in this manner there is constant immune surveillance against tumors. Failure to react with the new antigen results in cancer.

SELECTIVE VS. INSTRUCTIVE THEORIES OF THE IMMUNE RESPONSE

If, as we have said, there are cells which are able to produce antibodies of great specificity and there are cells able to react with tissues with an equal degree of specificity, it is very important to

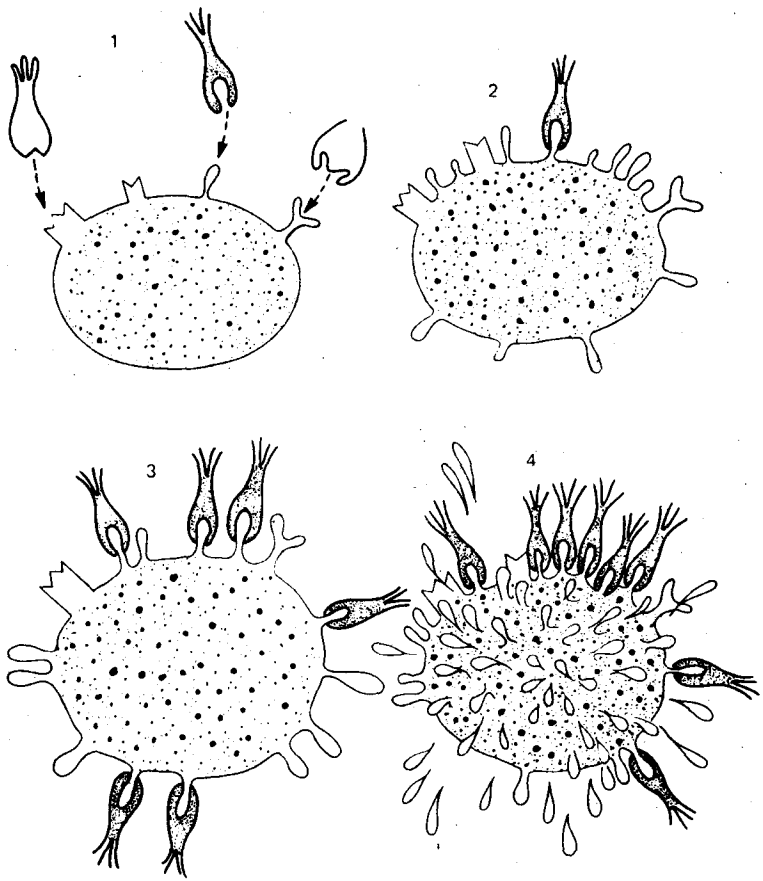
know how these cells interact with the specific antigen to initiate and carry out the processes. One school of current immunological thought has it that the elements of immunological specificity probably arose only in one form in the animal. That is to say, it is simplest to think that the manner in which an antibody molecule expresses its specificity must be the manner in which a lymphocyte expresses its specificity. Now if we accept this notion for the time being and argue that cell and product (antibody molecule) derive their specificity from a common mechanism, the next step in our reasoning is that the cell must have something like an antibody on its surface to react with antigen. The logic is pushed even further (on some experimental evidence which we shall look at in a later chapter) to say that the cells which will make antibodies of a given specificity have molecules of that antibody on their surfaces which act as *receptors* for antigen. Interaction between receptor and antigen somehow transmits a signal to the cell to produce more of the same antibodies and to divide. We know that the specificity of the antibody molecule is derived from one small portion of the peptide chain of the molecule; the argument thus runs that the genome of the cell producing the antibody has the genetic code for that particular sequence of amino acids which gives the molecule its specificity and that all the antibody molecules synthesized by that particular cell have that same sequence of amino acids and therefore that specificity. Once some fundamental assumptions are made, it really is an attractive hypothesis.

One prediction from the above theory is that there should be antibody molecules on the surface of lymphocytes to act as receptor sites for antigen. This turns out to be the case for B-cells (Chapter 5). One of the identifying characteristics of the B-cell is the presence of antibody, or more correctly immunoglobulin, molecules on the surface of these cells. But life is not all beer and skittles and the T-cell does not have nearly the quantity of immunoglobulin on its surface that the B-cell does. This fact is rather troublesome, and it has generated a good deal of controversy in recent years. The nature of the T-cell receptor is a fundamental problem in immunology and as of this time is still in doubt. One must therefore ask, if the T-cell has a receptor which expresses its specificity in a manner other than the manner of the immunoglobulin and the B-cell, do we now have to explain two separate mechanisms of specificity?

Whatever the nature of the receptor, the evidence seems quite convincing that there are lymphocytes reactive to a particular antigen present in the animal before it is challenged with antigen. When the animal is confronted with an antigen, the number of these specific

cells rises rapidly, almost astronomically. The question is how? Paul Ehrlich (1854 to 1915) developed the first useful theory to answer this problem. Ehrlich was one of the first to suspect that the mysterious substances (antibodies) in the serum after disease or immunization were produced by cells, and he formulated a theory which is frighteningly up to date considering that it was put forth over fifty years ago. (Perhaps this tells us something about up-to-datedness. More likely it tells us that genius sees problems in unusual ways, whether the time for that view has come or not.) Ehrlich postulated that a cell had *side chains* and that these side chains were the serum substances, i.e., the antibodies. Each cell had a set of side chains which was a reflection of the responses which the animal could make. Thus, animals make antibodies against tetanus toxoid, and so there was a side chain for tetanus toxoid. Similarly, animals make a response against the organism which causes pneumonia, so there was a side chain for that organism. In modern jargon we would say that a given lymphocyte (cell) had an antibody molecule as a receptor (side chain) for each of the antibodies the animal could make. Ehrlich envisioned the antigen reacting with the specific side chain, and as a result of this interaction the other side chains disappeared and the cell began producing only the side chain with the specificity of the antigen. These side chains left the surface of the cell and appeared in the serum, thus raising the level (or titer) of serum antibody against the antigen. This concept is diagrammed in Figure 1.

This very clever theory stood for awhile, but the great immunochemist, Karl Landsteiner (1868 to 1943), produced evidence which led to the abandoning of the side chain theory. Landsteiner synthesized organic compounds and tested their ability to induce antibody or to react with antibodies made against similar molecules. He would synthesize a small molecule which, when attached to a large one, gave rise to antibodies directed against the small molecule. The small molecule was called a HAPTEN; the larger one to which it was attached, a CARRIER (or "schlepper"). When Landsteiner introduced an NO_2 group onto a benzene ring to make nitrobenzene as the hapten, he found that he could get specific antibody directed against the nitrobenzene. When he introduced two NO_2 groups to produce the dinitrobenzene hapten, he found that he got a specific antibody directed against that molecule. Similarly, if he added sulfonic acid or arsonic acid to the ring, he generated specific antibodies against these compounds. Well, how can one visualize a cell with specific side chains, each chain specific for a given antigen to which the animal can respond, if one can go into the laboratory and synthesize a



1

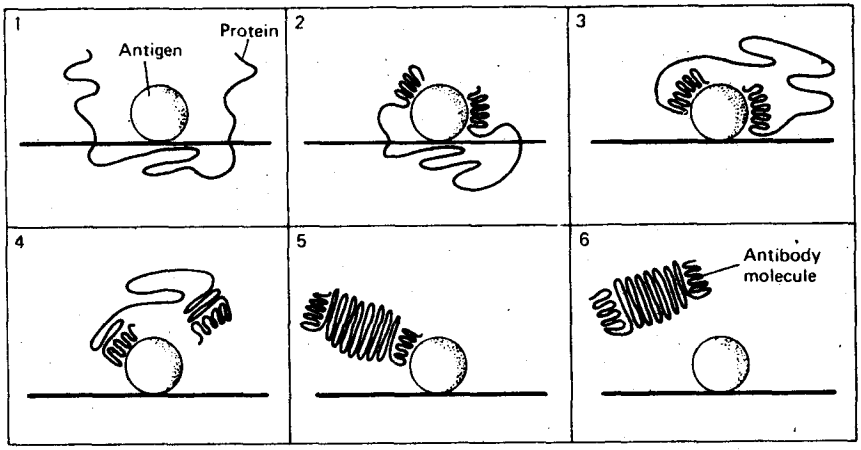
Paul Ehrlich's side-chain theory for antibody production. [From the original publication (1900). *Proc. Roy. Soc. B.* 66, 424.]

seemingly endless array of compounds? This discovery signaled the apparent end of the side-chain theory and introduced a new set of notions.

The side-chain theory really is a form of *selective theory*. Since the cell has the side chain, what is required is that it react with antigen and then more of the chains be produced. Thus the antigen selects the specific side chain. What followed was a novel move away from selective mechanisms to *instructive* theories of antibody formation. The leading proponents of these were Felix Haurowitz and then Linus Pauling in the 1940s and 1950s. Very briefly, the instructive theories

of antibody formation postulated that a cell which makes an antibody molecule is indifferent to the specificity of the molecule. The machinery for the synthesis of proteins was then not at all understood, and the cell was visualized as being able to spin out a protein molecule and then to introduce some imprint into the molecule in the final stages. This final fillip to a jaded molecule gave it the specificity for interaction. We know now that the final shape of a protein molecule is determined by the primary sequence of the amino acids and that this sequence is a reflection of the DNA which codes for that protein. The DNA is transcribed into RNA, which is then used as a template in translation to string together the proper amino acids. Since secondary and tertiary structure depends on primary structure, the specificity must be built into the genetic code. But in the 1940s these facts were not known, and the proponents of the instructive theories of antibody formation visualized that a small fragment of antigen got into the cell and altered the shape of the peptide chain as it was being synthesized. Thus, antigen instructed the cell as to the nature of the specificity of the molecule it was to produce.[2] This theory is diagrammed in Figure 2.

[2]The instructive theory of antibody formation had a great influence on early molecular biology. The theory was so attractive that attempts to understand regulatory phenomena in microbial systems were cast in instructive terms. Only when molecular biologists arrived at the "trinity" (DNA, RNA, peptide) was this idea no longer used in both molecular biology and immunology. I thank the historian of science Dr. Robert Obley for this instructive insight.



Linus Paulings's direct template theory. [From Pauling (1940). *J. Amer. Chem. Soc.* 62, 2643.]

As our knowledge of the mechanisms of protein synthesis increased and as it became increasingly difficult for the instructionalists to explain certain aspects of the immune response (the secondary response and tolerance, in particular), there was a growing uneasiness with the theory and a casting about for an alternative. Niels Jerne, probably as a result of the influence of the phage group on his thinking, evolved a new incarnation of the selective theories called the NATURAL SELECTION THEORY. Here is Jerne's reminiscence about his insight.[3]

'Can the truth *(the capability to synthesize an antibody)* be learned? If so, it must be assumed not to pre-exist; to be learned, it must be acquired. We are thus confronted with the difficulty to which Socrates calls attention in Meno (Socrates, 375 B.C.), namely that it makes as little sense to search for what one does not know as to search for what one knows; what one knows one cannot search for, since one knows it already, and what one does not know one cannot search for, since one does not even know what to search for. Socrates resolves this difficulty by postulating that learning is nothing but recollection. The truth (*the capability to synthesize an antibody*) cannot be brought in, but was already inherent.'

The above paragraph is a translation of the first lines of Soren Kierkegaard's "Philosophical Bits or a Bit of Philosophy" (Kierkegaard, 1844), By replacing the word "truth" by the italicized words, the statement can be made to present the logical basis of the selective theories of antibody formation. Or, in the parlance of Molecular Biology: synthetic potentialities cannot be imposed upon nucleic acid, but must pre-exist.

I do not know whether reverberations of Kierkegaard contributed to the idea of a selective mechanism of antibody formation that occurred to me one evening in March 1954, as I was walking home in Copenhagen from the Danish State Serum Institute to Amaliegade. The train of thought went like this: the only property that all antigens share is that they can attach to the combining site of an appropriate antibody molecule; this attachment must, therefore, be a crucial step in the sequences of events by which the introduction of an antigen into an animal leads to antibody formation; a million structurally different antibody-combining sites would suffice to explain serological specificity; if all 10^{17} gammaglobulin molecules per ml of blood are antibodies, they must include a vast number of different combining sites, because otherwise normal serum would show a high titer against all usual antigens; three mechanisms must be assumed: (1) a random mechanism for ensuring the limited synthesis of antibody molecules possessing all possible combining sites, in the absence of antigen, (2) a purging mechanism for repressing the synthesis of such antibody molecules that happen to fit to auto-antigens, and (3) a selective mechanism for promoting the synthesis of

[3]As an interesting aside, when Jerne wrote this reminiscence he was director of the Paul Ehrlich Institute in Germany. This seems rather appropriate.

those antibody molecules that make the best fit to any antigen entering the animal. The framework of the theory was complete before I had crossed Knippelsbridge. I decided to let it mature and to preserve it for a first discussion with Max Delbrück on our freighter trip to the U.S.A., planned for that summer. [Niels K. Jerne, *The Natural Selection Theory of Antibody Formation; Ten Years Later,* In *Phage and the Origins of Molecular Biology,* Cold Spring Harbor Laboratory of Quantitative Biology, 1966, p. 301.]

In his NATURAL SELECTION THEORY Jerne visualized that a cell was programmed to make only one specificity of antibody and that it did this even in the absence of antigenic stimulus. Thus there would always be a low level of antibody in the serum. When antigen was introduced into the system, this antibody would react with the antigen, and the antigen-antibody complex would find its way back to the cell which originally produced the antibody. The interaction of the antigen-antibody complex with the cell now stimulated the cell to divide, and there were thus many more of these cells after a short time, all producing antibodies of that one specificity.

F. M. Burnet (later Sir MacFarlane Burnet) modified this theory into the CLONAL SELECTION THEORY, the theory that most immunologists function under today and which will be assumed to be true in most of the thinking in this book. According to the clonal selection theory, a cell is programmed in its DNA to make one or at best a very few specificities. Antigen reacts with receptors at the surface of these cells, and this reaction constitutes a signal for the cell to divide. After several rounds of multiplication these cells which have been selected by antigen are the dominant specificity of the lymphocytes in the body, and the antibody they produce is found in high concentration in the serum.

This is essentially the state that the study of immunology has reached today. Virtually all practicing immunologists adhere to a clonal selection theory of one shade or another. All agree that the response has specificity and that there is roughly a cellular and a humoral aspect with varying degrees of overlap. How then does the whole thing work? How does the animal prevent reactions against itself? What controls the response so that it ceases at some point? All this and more make up the field of immunobiology, and this book will attempt to go into the major experiments which have provided the current thinking.[4]

[4]One of the leading philosophers of science, Thomas Kuhn, in his book *The Structure of Scientific Revolutions* argues that science moves forward by changing *paradigms.* A paradigm is a commonly held belief among scientists. It need not be correct, merely accepted. Kuhn argues that all experiments are really designed to prove the paradigms. When enough data is generated so that the paradigm begins to be

READINGS

Burnet, F. M. (1959). *The Clonal Selection Theory of Immunity*. Nashville, Tenn., Vanderbilt University Press. (The basis of the current paradigms.)

Burnet, F. M. and Fenner, F. (1949). *The Production of Antibodies*, Melbourne, Macmillan. (The idea of self-nonself is beautifully developed here.)

Jerne, N. K. (1955). The natural selection theory of antibody formation, *Proc. Nat. Acad. Sci.* **41,** 849. (A critical idea paper that started the modern era.)

Jerne, N. K. (1967). Waiting for the end, *Cold Spring Harbor Symposium on Quant. Biol.* **32,** 591. (The state of the art in 1967 when it looked like the end was near.)

Landsteiner, K. (1936). *The Specificity of Serological Reactions*, Springfield, Ill., Thomas. (A classic.)

Lederberg, J. (1959). Genes and antibodies, *Science,* **129,** 1669. (A brilliant analysis by a nonimmunologist who is one of the founders of modern genetics.)

Pauling, L. (1940). A theory of the structure and process of formation of antibodies, *J. Am. Chem. Soc.* **62,** 2643. (An early, clear exposition of the direct template idea.)

GENERAL TEXTS

Eisen, H. (1974). *Immunology,* Hagerstown, Md., Harper & Row. (A masterful covering of all aspects of immunology.)

Fudenberg, H. H., Stites, D. P., Caldwell, J. L., and Wells, J. U. (1976). *Basic and Clinical Immunology,* Los Altos, Calif., Lange. (A good text with a clinical orientation.)

Good, R. A., and Fisher, D. W. (eds.) (1971). *Immunobiology,* Sunderland, Mass., Sinauer Associates. (A very good series of papers on many aspects of the immune response.)

Kabat, E. A. (1968). *Structural Concepts in Immunology and Immunochemistry,* New York, Holt, Rinehart and Winston. (A thorough covering of the structural basis of immunoglobulin and antigen binding.)

Kuhn, T. (1970). *The Structure of Scientific Revolutions,* 2nd ed., University of Chicago Press. (An extremely important book which presents the argument of changing paradigms.)

FOOTNOTE 4 (*Continued*)

less universally accepted, a new paradigm takes its place. This view of science is contrary to that of Karl Popper, who argues that scientists actually set out to disprove ideas. But whether Kuhn or Popper is more correct is not our prime concern. I have used Kuhn's notion of paradigms in selecting experiments to illustrate the developing thoughts of modern immunobiology. Regardless of *how* paradigms change, the fact remains that they *do* change, and I have tried to show the evidence which caused immunologists to adopt new paradigms.

Mazumdar, D. M. H. (1975). The purpose of immunity: Landsteiner's interpretation of the human isoantibodies, *J. Hist. Biol.* **8**, 115. (A very readable article by an historian showing the changing ideas of the study of the immune response.)

Roitt, I. M. (1971). *Essential Immunology,* Oxford, Blackwell Scientific Publications. (A readable overview of immunology with an orientation toward antibody and antibody-mediated hypersensitivity.)