William R. Clark

THE EXPERIMENTAL FOUNDATIONS OF MODERN IMMUNOLOGY

Second Edition

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SECOND EDITION

WILLIAM R. CLARK

Department of Biology
and the
Molecular Biology Institute
University of California, Los Angeles

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Preface

My feelings after finishing the first edition of this book were largely ones of relief. I vastly underestimated the amount of effort required simply to get that much information together and into print. But preparing a textbook can also be a tremendous learning experience; my own immunology lectures have certainly profited from having to research each fact thoroughly before committing it to potential scrutiny by every real and aspiring immunologist in the English-speaking world.

The rapid accumulation of new information since the first edition was sent to press provides me with an excuse to prepare a second. I am grateful to all of my colleagues who generated this information, since it gives me an opportunity not only to update the previous text but to smooth out, reorganize, and better integrate some of the material unaffected by recent progress. I am also grateful to the many individuals who communicated their ideas about the text to me, and who pointed out errors of fact or omission.

An important pedagogical point that I omitted in the preface to the first edition is that I do not consider this or any other text a substitute for reading the literature. That is why, for example, people carrying out particular experiments or lines of research are identified by name, and why key references are given at the end of each chapter. In a rapidly developing field like immunology, a course or a text can at best teach a language and give a general background of fact enabling the student to begin reading the primary literature on his or her own. Many of the most exciting stories contained in these chapters are left hanging as if in mid-sentence. If you are curious about the outcome, look up the experimenter in the Author Index of Chemical Abstracts or Biological Abstracts, check out their latest papers, and see how the story turned out!

I am again indebted to a number of people for direct help in preparing various parts of this text. Eli Sercarz has continued to provide valuable and critical input. Amy Percy contributed comprehensive information on the immune response to parasites. Randy Wall again provided valuable guidance for the section on immunoglobulin genetics, and Lorraine Flaherty made useful comments on the section on the Tla system in mice.

WILLIAM R. CLARK

Preface to the First Edition

This book was written for two reasons. First, I want to provide a comprehensive arrangement of information about immunology that can serve as a foundation for those interested either in a career in medicine or in basic research. I think both directions can best be served by developing in the student a strong sense of the experimental foundations of contemporary immunological concepts. My second purpose is to convey to all students my feeling of genuine delight and excitement in the growing field of immunology. Immunology is a truly universal biological science, cutting across and actively influencing almost every other area of contemporary biological research.

The text presented here is based essentially on the immunology course taught at UCLA. It is mostly a senior-level course, and presumes a background consisting of at least one university-level course in biochemistry, in genetics or molecular biology, and in cell biology. In our experience, about a quarter of each class is composed of first- or second-year graduate students who, as undergraduates, did not have an intensive course in immunology.

Virtually all of the information about non-disease-oriented immunology has accumulated in the past 15-20 years, and this information has been in some ways unstable, often undergoing radical changes and about faces in a remarkably short time. This has led to a tendency to generate immunology textbooks that either ignore completely the enormous ferment in current immunological research, or that emphasize current research and ignore entire areas of immunology that are not only vital to a balanced appreciation of the field but that in fact offer important background for and insights into contemporary research questions. The latter type of book has tended to be rather brief and quickly produced, perhaps reflecting a conviction that the information conveyed may be of relatively short-lived value. In writing this text, I have taken the position that it is time at least to attempt to write a more comprehensive treatise on the "postserology" phase of immunology, and to begin a collation and assessment of those experiments that have had a major influence on current immunological thought. I have thus attempted to achieve a balance between presentation of the experimental basis of the "classical," generally well-accepted concepts of immunology, and exposure to the kinds of experiments and conceptual thinking that are contributing in a

significant way to current immunological research. There is always the problem with the latter that the information may become stale before the book reaches the shelf. I have therefore tried to make it clear that the information presented in such cases is not to be taken as hard fact; it is presented in order to indicate the directions in which current immunology research is headed, and to enable the student to plunge into the immunology literature and share in the excitement of watching new ideas develop.

Not all of the information in this text is presented from an experimental point of view: Chapters 2 and 5 are simply expository. Certain topics have been deliberately omitted as being too esoteric or vague for an introductory text—network theory is one example. Other topics (e.g., tumor immunology and detailed serology) have been omitted because while they are clearly important in their own right, their contribution to understanding fundamental principles of how the immune system works is minimal. Not everything can or should be covered in a first course; presumably these and other topics will be covered in advanced immunology or special topics textbooks and courses.

Like all authors of technical books, I would be delighted to have errors and omissions brought to my attention. And like all authors, I am indebted to many people, only a few of whom can be acknowledged here. Eli Sercarz has been a constant source of information and, more importantly, a strong and critical proponent of the use of key experiments as an approach to teaching. Randy Wall contributed excellent advice and assistance in formulating the section on immunoglobulin genetics. Harden McConnell provided a haven for writing a good portion of this book, and inspired me to explain immunology in terms that intelligent scientists of all persuasions can understand. Finally, I express my deep appreciation to Mayo Uchiyamada, who assisted me in the preparation of the original manuscript; without her organization, persistence, and willingness to argue, this text would have been at least another six months in appearing.

W. R. C.

Contents

Chapter One

INTRODUCTION

One of the fascinations of immunology is that it overlaps with, contributes to, and is enriched by such a wide variety of disciplines. Immunology, perhaps to the discomfiture of scientists in less eclectic fields, seems continually to be making contributions to areas as diverse as protein structure, cell differentiation, membrane biology, and eukaryote genetics. Yet at the pedagogical level, immunology has tended to be a rather isolated subject. Most colleges and universities do not have a separate undergraduate course in immunology; even in medical schools immunology is usually buried somewhere in a microbiology course. While most students of biology are vaguely familiar with at least the concept of an antibody, little of the structure or function of the immune system per se is included in lower-division preparatory courses. And since a study of immunology requires the development of a fairly specialized vocabulary, few students develop a casual interest in it on their own.

The requirement for a new scientific vocabulary, or at least the equivalent of a new dialect, also makes immunology somewhat difficult to teach. No matter where one chooses to start, initial progress is bound to be slow until language develops and fundamental concepts become familiar. Thus it is well to spend some time at the beginning of a course or a textbook in trying to organize key ideas and to define important terms. In what remains of this short chapter we do just that. Every idea touched upon lightly here will be expanded and expounded upon many times over in this text. But it is a good idea to have at least a look at the forest before becoming lost among the trees.

THE CONCEPT OF IMMUNIZATION

Most animals are born with virtually no immune protection. A few antibodies cross the placenta from the mother, and in some species antibodies may also be present in the colostrum (milk secreted immediately after birth). Such importation of ready-made

immune components from another person or animal is a form of *passive immunization*. The perinatal acquisition of antibodies from the mother is highly variable from species to species, and at best provides protection for only a few months after birth. Endogenous immune defenses are built up gradually as the newborn is exposed to environmental antigens, principally microbial. This type of acquisition of immunity is one form of *active immunization*. Another form of active immunization involves the deliberate introduction of foreign matter or antigen into a host animal in order to provoke an immune response prior to natural encounter with the antigen. This is the process commonly referred to as vaccination.

The validity of vaccination in conferring protection against subsequent infection was first put on a firm scientific basis by Edward Jenner at the end of the eighteenth century. However, references to the efficacy of some form of vaccination in prevention of smallpox have been discovered in ancient Arabic and even earlier Chinese medical manuscripts. The concept of vaccination against smallpox was introduced into England in the early 1700s by the wife of the British ambassador to Turkey, whose daughter was actually the first person in England to receive a smallpox vaccination (1718). Such vaccinations usually consisted of scratching small amounts of dried or powdered scab from healed smallpox lesions into a small wound on the skin. As can be imagined, there was considerable risk associated with this procedure. Jenner's major contribution was to recognize that persons exposed to cowpox, a relatively mild malady in humans, were protected from smallpox. Specifically, he observed that milkmaids, who often had pockmarks on their hands as a result of cowpox, seemed never to contract the more deadly smallpox even though fully exposed to it. In 1796 he vaccinated a young boy with cowpox, and then later deliberately exposed him to pus from a smallpox lesion. The boy failed to develop smallpox, providing Jenner with a basis for extending his studies to others. Within a few years the validity of vaccination was accepted by most of the medical and scientific community, although its complete acceptance by the general population in England and elsewhere came about only in the middle of the nineteenth century. Inquiry into the means by which vaccination conferred protection against subsequent infection was the beginning of the science of immunology.

Little progress was made in applying the knowledge gained in Jenner's experiments until the second half of the nineteenth century, principally because the biological basis of infectious diseases was completely unknown. Further progress in the field of immunology was thus dependent to a considerable degree on developments in microbiology. By far the most influential figure in the development of both fields was Louis Pasteur. In the course of his studies on the characterization of microbes, he discovered that certain of them could be rendered noninfectious while nevertheless retaining their ability to confer immunological protection. He found, for example, that anthrax bacilli grown at 42°C (instead of 37°C) could no longer infect sheep, but could confer protection against subsequent exposure to fully active bacilli. Pasteur was thus the first to introduce the concept of immunization with attenuated microorganisms. His pioneering work, which also included the development of vaccines (a term actually proposed by Pasteur) for cholera and rabies, opened the way for nothing short of an

explosion in the management of infectious diseases. It is difficult to imagine any single contribution, perhaps other than the development of antibiotics, which has had such a profound effect on human health.

Although Louis Pasteur made immunization a highly practical science rather than a vague practitioner's art, the biological basis of the immune response remained unknown for many years. One of the first proposals for a biological mechanism came from Elie Metchnikoff. Working in France and Russia in the 1880s, he observed in several invertebrate species that foreign objects, including microorganisms, were often surrounded by free-living, motile cells that subsequently ingested and destroyed the invading material. This discovery created considerable ferment and quickly led to formation of a school of thought, headed by Metchnikoff, that proposed this phenomenon (called phagocytosis) as the basis for the immune reaction. However, within a few years this proposal was strongly challenged by several scientists, first by an American, George Nuttall (1888), and later, and perhaps more forcefully, by Emil von Boehring (1890). Both of these workers, as well as others, showed that the noncellular, nonclotting elements of blood (serum) from previously immunized animals contained factors that were either directly and specifically lethal to the microorganisms used for the immunization, or capable of neutralizing their toxins. The debate between the advocates of the cellular and humoral theories of the immune response raged vigorously, and rather acrimoniously, until the turn of the century. The English physician, Almroth Wright, showed in 1903 that the immune attack on microorganisms actually involved both humoral and cellular elements. He observed that certain humoral factors, which he called opsonins, in some way rendered bacteria more susceptible to ingestion by phagocytic cells. Opsonins were soon shown to be identical with serum antibodies, which, together with complement, could directly kill bacteria. In such cases phagocytes ingested the killed microorganisms, as well as damaged host cells, at the site of the infection.

The history of immunology beyond the resolution of the apparent humoral-cellular dichotomy is woven throughout the chapters of this book. The crowning achievement of the immunization process was probably the total eradication of smallpox. In the first half of the twentieth century, 2–3 million new cases were reported annually. The last case of smallpox in the United States was seen in 1949, and the last verified case in the world was reported in Somalia in 1977. In fact, most western nations no longer recommend immunization for smallpox; the risk of immunization is considered greater than the possibility of exposure to the disease in its virulent form. It seems fitting that the very problem that stimulated development of the immunological approach to control of infectious diseases has been the first to be totally controlled by that process.

The antibodies that are the basis of the humoral protection conferred by immunization were not to be defined at the molecular level for nearly 50 years after their discovery, although a good deal would be learned about their properties by the early 1900s. Knowledge of the cellular basis of the immune response beyond the phagocytic process has really been developed only in the past 15 years or so. Even today we understand the genetic, molecular, and cellular parameters of immune responsiveness

only in broad outline. But enough information has been gathered so that the major questions to be resolved are at least defined, and it is clear that the field of immunology will be an active and exciting one for many years to come.

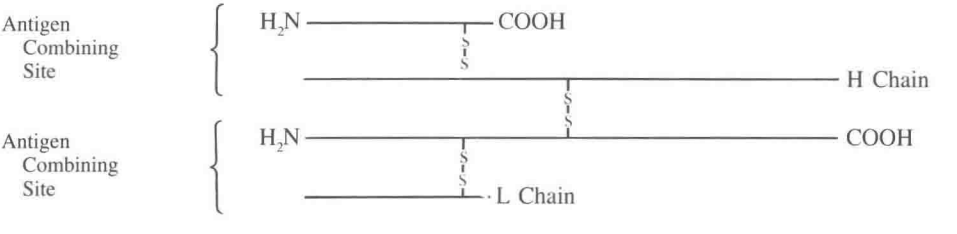
WHAT IS THE IMMUNE SYSTEM, AND WHAT DOES IT DO?

The immune system is actually the body's third line of defense against invasion by foreign substances. The first line of defense is the skin and the various other epithelial linings of those portions of the body in contact with the environment, which present a physical barrier to intrusion by foreign matter. The second line is the battery of nonspecific host mechanisms that compromise the survival and growth of pathogenic microorganisms (pH; cell sloughing; ciliary sweeping; sneezing and swallowing; "genetic" resistance; etc.). These mechanisms are sometimes collectively referred to as innate immunity.

What is meant by "foreign"? Under most circumstances, foreign means anything organic that is not coded for by the host organism's DNA. While this definition will suffice in nearly all cases in nature, some important exceptions exist or can be created, and these are discussed at various points in this text. It is quite clear that the major selective force behind the evolution of the immune system is the need to combat invasion by foreign microorganisms such as bacteria, yeast (molds), and viruses. As we have already seen, the study of resistance to infectious disease was the major impetus for studying and characterizing the immune system. Such studies, however, now encompass only a very small part of the general field of immunology, the major portion of current efforts being devoted to analyzing the genetic, molecular, and cellular facets that underlie all immune reactions.

There are two ways in which the immune system can deal with invasion by a foreign substance. One way is to produce a globular, circulating glycoprotein called an *antibody*. An antibody molecule recognizes and attaches to the foreign substance in a very specific way. This reaction of the antibody with the foreign substance, or *antigen,* triggers a series of events leading to elimination of the antigen from the system. This type of immune response is called a *humoral immune response*. It can be directed toward a wide range of foreign substances, both molecular and cellular.

Antibodies (Ab), which circulate freely in the blood and lymph, are produced by a type of white blood cell called a *B lymphocyte*. All antibodies are variations of the basic four polypeptide chain immunoglobulin (Ig) structure:



This structure is composed of two light (L) chains, of molecular weight about 25,000, and two heavy (H) chains of about 50,000 MW. The chains are held together by disulfide bonds. This unit Ig structure has two *antigen-combining sites*. Each site is formed by the NH_2-terminal portions of adjacent H and L chains.

The second way in which the immune system deals with invasion of the body by foreign material is through a *cell-mediated immune response*. In this case, a specific type of lymphocyte, called a *T cell,* recognizes and binds to the antigen, again leading to the ultimate elimination of the latter from the system. These lymphocytes are distinct in a number of ways from those producing antibodies, (*B cells*). The T-cell reaction is principally, although not exclusively, directed toward antigens associated with the surface of pathological cells such as tumor cells, virally infected cells, etc. The T cell recognizes the aberrant cell through a receptor molecule on its surface, which has antibodylike properties but is probably not classical antibody per se. In the case of both humoral and cell-mediated cytotoxic reactions, foreign matter (plus expired host cells) may be removed from the reaction site by *phagocytes,* cells which literally "eat" other cells, bacteria, macromolecular complexes, and even inert inorganic particulate matter.

FUNDAMENTAL CONCEPTS IN IMMUNOBIOLOGY

Antibody Specificity

One of the cardinal features of the reaction of antibody and antigen is the specificity of the reaction. An antibody produced in response to one antigen will cross-react poorly, if at all, with most other antigens.

Antigens are defined as substances, both cellular and molecular, capable of being bound by either an antibody or an immune lymphocyte. (When a whole cell is referred to as an antigen, we are really talking about membrane-associated macromolecules as antigens.) Any organic molecule (i.e., with a molecular structure based on carbon) is a potential antigen. It will be obvious from a cursory inspection of the antigen-combining site of an Ig molecule that only a small portion of a macromolecular antigen can actually interact with an antibody molecule. Thus, while an entire macromolecule, cell, or microorganism may be referred to as an antigen, only those discrete portions of the macromolecule that interact with the antigen-combining site, called antigenic *determinants* or *epitopes,* actually trigger the immune response.

A necessary distinction must be made between an antigen and an *immunogen*. An immunogen is defined by its ability to provoke an immune response (either cellular or humoral). While all immunogens are also antigens, in that they can combine with antibodies, not all antigens are immunogens. The distinction seems to be primarily one of size. Very small molecules (on the order of size of an amino acid, or a single sugar molecule, or a phenol ring, for example) cannot by themselves induce an immune response. However, if they are coupled to a larger molecule (called a *carrier*), they then become essentially an antigenic determinant or epitope, and can be perceived

by and responded to as immunogens by the immune system. Such small molecules, which by themselves are antigens but not immunogens, are often called *haptens*.

The basis for antibody-antigen reactions is qualitatively similar to the basis for enzyme substrate reactions in that they involve energetically favorable interactions between chemical functional groups in the antigen-combining site, and on the antigenic determinant. Thus in reality an antibody produced in response to one antigen could cross-react with any other molecule that had an antigenic determinant of identical or very similar structure. The low degree of cross-reactivity generally observed simply reflects the enormous structural diversity of biological molecules. The important point to keep in mind is that it is not the case that each antibody is made for one—and only one—antigen.

Antibody Diversity

One of the most puzzling features of the immune response, from the point of view of early researchers in the field, was the fact that almost any organic compound known could apparently elicit an antibody that reacted with it in a reasonably specific way. This implied the existence of a very large number of possible antibody molecules. The problem was seemingly made even more complex by the work of Karl Landsteiner in the 1930s. He showed that by introducing relatively slight chemical modifications to haptenic groups, he could induce antibodies that were barely cross-reactive. For example, antibody produced in response to *p*-aminobenzoic acid interacts almost not at all with *m*- or *o*-aminobenzoic acid. And other organic chemists-turned-immunologists demonstrated that almost any compound they chose to synthesize could elicit the production of a very specific antibody. Thus molecules that had never before existed on earth, and which obviously could not have played any evolutionary role in selecting antibodies, could nevertheless trigger the production of a specific antibody. The basis for this property of immune responses remained a complete mystery until the middle of the present century.

Memory

A characteristic feature of the immune system, with which nearly everyone is familiar, is memory. It is this feature that is referred to when we say someone is "immune" to a particular disease, for example. The first time an individual is exposed to a given pathogen, the resulting disease may progress to the clinically detectable stage or beyond, in some cases even resulting in death. In most instances the immune system will neutralize the pathogen or the microorganism producing it, and symptoms of the associated disease will disappear. Upon subsequent (secondary) exposure to the same pathogen, the immune response will be mobilized much more rapidly, and will manifest itself much more strongly, than the original (primary) exposure. Very often, the associated disease will not even reach the clinically detectable stage, or at best will

cause only mild symptoms. Memory can be generated to virtually any antigen, and is characteristic of both the humoral and cell-mediated branches of the immune system. Persistence of the memory state is variable from individual to individual, and also varies among different antigens, but can be quite long—up to 10 years in humans. Any theory attempting to explain the operation of the immune system must be able to account for this feature of immune responsiveness.

Tolerance and Self/Non-Self–Discrimination

The phenomenon of tolerance can be thought of as an expression of negative immunological memory: As a result of previous exposure to a particular antigen, the subsequent immune response is reduced or totally abrogated. Like memory, tolerance can be demonstrated for both the humoral and cell-mediated branches of the immune system. For the most part, tolerance is an experimental phenomenon, generated in laboratory animals by manipulating the form, dose, or timing of antigen administration. There are, however, examples of tolerance in nature. Among outbred populations such as humans, a skin graft from a child to its mother will usually persist longer than a child-to-father graft, suggesting some sort of transplacental tolerization of the mother to paternal antigens on the fetus during embryogenesis. Experiments with inbred strains of mice have shown that a female mouse repeatedly made pregnant by a male (or males) of a genetically different strain may develop significant tolerance toward skin grafts from mice of the male partner strain. This observation also lends support to the notion that mothers and fetuses may have substantial degrees of histological communication either normally across the intact placenta or briefly at birth at the moment of placental rupture.

The most obvious and important natural expression of tolerance of course is the ability to discriminate self from non-self, and it is likely that all other forms of natural or experimental tolerance are simply expressions of this very central feature of immune responsiveness. The experiments of Landsteiner showed that the immune system can respond to almost any conceivable organic molecule—why then does it fail to respond to the organic molecules of which self is composed? Obviously this cannot be allowed to happen, but how is it prevented? Ray Owen reported an observation in 1945 that gives some clue to at least the timing of this event. Genetically distinct cattle twins that share a common blood supply during embryogeny will, as adults, each carry two genetically distinct sets of blood cell elements. The adult uses both sets of blood elements equally well, and neither is immunologically rejected. Were an exchange of blood elements carried out between two normally developed adults of the same two genotypes, rapid, mutual, and vigorous immunological rejection would occur. This was the first suggestion that foreign antigens seen by the immune system during embryological development might be perceived as self, whereas elements encountered for the first time after birth would be considered foreign. In general, this turns out to

be true, although as we will see in Chapter 8, the mechanisms by which this occurs are still far from clear.

Theories of Antibody Production in Response to Antigen

We have already pointed out that the great clinical importance of immunology provided much of the early impetus for its development as a field of study. But there were always a few individuals who perceived immunology as a science in its own right, with its own unique challenges and problems to be resolved at both the theoretical and experimental levels. Foremost among the problems requiring a theoretical framework was the question of antibody diversity. How is a specific antibody produced in response to a seemingly infinite variety of molecules, some of which have no natural phylogenetic history of interaction with immune systems? A second but equally important question concerned the immunological distinction between self and nonself. If the immune system can produce antibodies in response to almost any known organic molecule, why does it fail to do so in response to self-molecules? Almost any molecule taken from one organism and injected into another will provoke an antibody response, and is thus clearly immunogenic; why then is no antibody made against such molecules in their own immunological environment?

Paul Ehrlich was the first to propose, in 1894, a theory to account for the cellular origin and diversity of antibody. He suggested that in order for a cell to take up nutrients, it must have specific cell surface receptors for each nutrient. These receptors, he believed, would combine by standard chemical means with defined portions of the nutrient molecules. Thus each receptor would be specific for one or at best a very few closely related nutrients. A mature organism would have a wide range of receptors, capable of combining with almost anything that could potentially serve as a food source. Following a suggestion of Weigert, Ehrlich subsequently proposed that when nutrient materials (antigens) combined with the surface receptors (antibodies), the cell bearing these receptors (or perhaps "sister cells," bearing the same receptors) would overproduce copies of these receptors-antibodies and shed them into the serum. This theory accounted for the existence in serum, and chemical specificity, of antibody in response to antigenic stimulation.

A key element of Ehrlich's hypothesis was that all possible antibody molecules, in terms of antigenic specificity, preexisted in the host independently of exposure to or contact with antigen. This notion became increasingly difficult to accept as Karl Landsteiner and his colleagues began to publish their work on antibody specificity in the second quarter of the twentieth century. They found that highly specific antibodies could be induced by virtually any chemical, even by molecules that had never existed until manufactured for the very first time in the chemist's laboratory! The dilemma this posed was stated clearly by Stuart Mudd in 1932: "How may it be conceived that the animal body can thus develop substances possessing specific correspondence with the numberless proteins found in nature or capable of preparation in the labo-