PRINCIPLES OF
Immunology

Edited by

Noel R. Rose

Felix Milgrom

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Preface

Only a few years ago, a textbook of immunology for students of medicine and other health sciences, graduate students of biomedical sciences, and clinicians would have had little justification. It is not that immunology is a new science; on the contrary, the readers of Chapter 1 will find that it is among the oldest of the natural sciences basic to medicine. Immunology has had, rather, the status of a collaborator of microbiology, pathology, biochemistry, clinical medicine, and other disciplines. This integration with other sciences has been a strength and, at the same time, a weakness. It has meant that immunologic approaches have been brought to bear on a great many biologic and medical problems, giving an extraordinary richness and versatility. Accordingly, immunology has enjoyed the stimulation of intimate links with other rapidly growing areas of science.

Yet too often biologists and physicians, unfamiliar with the basic principles of immunology, have failed to understand their real potential benefits. Young investigators have sometimes been more aware of techniques than principles; health practitioners have applied the methods of immunology without understanding their broader implications.

At the urging of the late Ernest Witebsky, Distinguished Professor of Bacteriology and Immunology, a Center for Immunology was chartered by the Trustees of the State University of New York in 1967 to meet the special needs of a science that had not yet found its proper academic setting. With responsibility to foster research and training in all aspects of immunology, it brought together immunologists in many different departments of the State University of New York at Buffalo, its affiliated hospitals, and the related Roswell Park Memorial Institute. The creation of this book was long the dream of Ernest Witebsky, and it culminates several years of close collaboration of the Buffalo group of immunologists. Dr. Witebsky's untimely death on December 7, 1969, cut short his participation.

In organizing this book, the editors have attempted to attain certain goals. The text is graduated, so that the beginning student can systematically build concept upon concept, chapter by chapter. Yet each chapter is entire in itself, thus allowing the more experienced reader to review a particular subject in a meaningful way. The editors have tried to avoid duplication by extensive cross-references among chapters, but have not hesitated to treat the same subject from several different viewpoints in various chapters to

attain a more balanced or comprehensive presentation. The character of this book precluded any extensive list of references, but all chapters end with a succinct bibliography, as a guide to further information on each subject.

After a first chapter designed to give the uninitiated reader an overall perspective of immunology, the book is divided into two roughly equal parts: the first 15 chapters treat the basis of immunology, and the last 13 chapters pertain to applications of the subject. The book aims at providing the fundamentals of immunology for medical, dental, and graduate students, as well as for physicians and other biomedical scientists who wish to be brought abreast of this important and rapidly evolving discipline.

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1

Historic Roots and Scope of Immunology*

ERNEST WITEBSKY, FELIX MILGROM, AND NOEL R. ROSE

Immunization

In common usage the term "immunity" denotes specific protection. It comes from the Latin adjective *immunis* meaning "free from duty." It was observed even in ancient times that people recovering from certain infectious diseases are resistant and that this resistance or immunity is characterized by its specificity. Recovery from an infectious disease was accompanied by immunity to that particular disease only, and not to any other disease.

History records many early attempts to induce immunity by artificial means. Emanuel Timoni, a native of Italy who practiced medicine in Constantinople, first described at a Royal Society meeting in 1713 the practice of inoculation for the smallpox which had then already been in successful use in Turkey for 40 years. The inoculation was made with small amounts of fresh pus from pustules of young smallpox patients, into several little wounds in the arms or legs of normal subjects, with the help of a needle. This procedure almost invariably gave rise to a very mild form of the disease, leaving no scars or pits on the face, and it conferred lasting immunity. Lady

* Portions of this chapter are modified from the following article: Witebsky, E.; Milgrom, F.; and Rose, N. R.: An immunologist's vade mecum. *Karger Gazette*, No. 13, 1966. By permission of S. Karger, Basel/New York.

Mary Wortley Montagu, the wife of the British ambassador to Turkey, described the practice in 1717, and she subsequently was instrumental in making the method popular in England. In 1798 the English physician Edward Jenner, having noted earlier in his medical practice in Gloucestershire that it was firmly believed locally that dairy workers who had contracted cowpox were thereby protected from a subsequent attack of smallpox, published the results of experiments he had conducted in 1796. He had procured material from a case of cowpox and injected it into several human volunteers. Cowpox (the Latin word is *vaccinia*, from *vacca*, "cow") usually produces only a localized lesion in humans, but it conveys immunity against smallpox. Jenner verified this immunity by challenging his volunteers by inoculating them with smallpox pus. Jenner's important contribution was based on careful clinical observations. He had discovered, without realizing it, the important principle of inducing immunity by inoculating humans with a naturally occurring virus of a low pathogenicity that establishes a state of immunity similar to that induced by the fully virulent infectious agent. Pasteur, sixty years later, used physical and chemical means to attenuate virulent organisms. He cultured anthrax bacilli at temperatures higher than body temperature, 43° to 44° C. The longer anthrax bacilli were grown at this temperature, the greater was the loss of their pathogenic potency. The word "vaccines" was coined by Pasteur in honor of Jenner to denote all microbial preparations used for induction of specific protection against infection. Pasteur's further work on the preparation of a vaccine against rabies, by combining the principle of animal passage with the physical means of drying infected spinal cord for various lengths of time, still constitutes a classic experiment in immunology.

According to the investigations of Salmon and Smith, not only living but also killed microorganisms can induce immunity. Thus, today, killed suspensions of causative agents of various diseases are used on a large scale to immunize against such important diseases as typhoid fever, typhus fever, whooping cough, plague, Asiatic cholera, and many others. This subject is discussed in depth in Chapter 21.

The discovery of the diphtheria exotoxin by Roux and Yersin proved to be another milestone in the understanding of the nature of infectious diseases. All the systemic clinical manifestations of diphtheria could be explained by the action of the soluble exotoxin rather than by the invasive properties of the diphtheria bacilli. Therefore, artificial immunization against diphtheria and some other diseases with a similar pathogenetic mechanism must be directed against the exotoxins. This topic is discussed in greater detail in Chapter 3.

On standing, toxic filtrates gradually lose their toxicity without losing their antigenicity, a phenomenon attributed by Ehrlich to the formation of toxoid molecules. Many different chemicals were added to toxins to accelerate the transformation of toxin molecules into toxoids until Löwenstein in Austria, and Ramon in France, found formaldehyde to be a most suitable

agent for that purpose. In this way it is possible to detoxify toxins completely without interfering with their antigenicity. Toxoids are used today on a large scale to produce immunity against all infectious diseases in which the immunity is antitoxic in nature rather than antibacterial.

Reviewing, then, the different principles used for active immunization against microbial diseases, we should basically differentiate between living but attenuated agents and lifeless immunizing material. In the first instance we actually induce an infection that is generally mild in character. In addition to smallpox, this principle is used for such diseases as yellow fever and poliomyelitis, where the virus is attenuated by propagating it in cell cultures. Of course, an infective microorganism is required to initiate immunization, but the resulting immunity is usually rather potent, practically as effective as that which accompanies the recovery from the disease proper. In contrast, immunization with killed bacteria or viruses, based on a series of three or four injections, does not require an infectious agent, but is frequently not as effective or as lasting as that elicited by living, attenuated agents. However, we should not underestimate the solidity of the state of immunity produced by inoculation with toxoids, such as diphtheria or tetanus toxoid.

The natural resources available to the host in its fight against infections were viewed in different ways by two different schools. Metchnikoff and his students at the Pasteur Institute discovered the phenomenon of phagocytosis, which they considered the underlying principle of natural and induced resistance. The other school of thought in those early days of immunology, of which Fodor, Buchner, and Nuttall were proponents, considered the bactericidal property of normal blood serum the most important weapon in the defense against infections. The discovery by Behring and Kitasato that serum was the site of antitoxic immunity against diphtheria opened up a new field and led to the introduction of serum therapy. The first Nobel prize in medicine was given to Emil von Behring for this work. Pfeiffer and Issaeff, and Gruber and Durham, recognized that bacteria, when injected into animals, also elicit the formation of potent antibodies demonstrable by lysis or agglutination. Finally, Wright and Neufeld combined the cellular and humoral concepts when they noticed a profound increase in the phagocytic activity of the immunized individual due to the production of phagocytosis-enhancing antibodies or opsonins (see Chapter 7).

Two basic methods of immunization have become apparent. The first one is that of *active* immunization, in which microorganisms or their products are used to elicit antibody formation. The second principle takes advantage of the antibodies produced in the actively immunized host by transferring his serum to normal individuals. It is called *passive* immunization because the individual protected in this way has done nothing himself to produce the state of immunity. These two types of immunity should be sharply differentiated. In the case of active immunization it often takes weeks for antibodies to appear, whereas the passive transfer of the serum of an actively immunized

donor into a normal recipient conveys immunity immediately. As to the duration of these two forms of immunity, the active type of protection remains potent for many months and even years, in contrast to the passive form of immunization, which fades out a few months after transfer of the immune serum. In common parlance, the term "vaccine" denotes a substance available for active immunization, and the word "serum" is used in referring to the matter used for passive immunization. The terms should never be used interchangeably, since they involve two quite different principles of immunization.

Antigens and Their Reactions

Bordet's discovery of the antigenicity of harmless substances, such as foreign red blood cells or milk, required the development of a new concept of the nature and meaning of antibody formation. It became apparent that antibodies are not only produced as a defense mechanism against invading pathogenic organisms or their toxic products but are the response of the host to the introduction of any kind of foreign antigenic material, especially foreign proteins. Antigens usually are not capable of eliciting antibody production when given orally but only if injected parenterally, thus avoiding the enzymes of the intestinal tract. In this latter case another biologic principle, antibody production, is called on to preserve the injected individual's integrity. These antibodies, in lieu of the enzymes, combine with the material injected and aid in eliminating it as fast as possible. Therefore, it becomes obvious that the production of antibodies is a general biologic phenomenon rather than a specialized mechanism designed to protect against infection. As a matter of fact, repeated injections of otherwise harmless material, such as milk or blood serum, can produce considerable harm under certain experimental conditions. Antibody production means not only protection but also hypersensitization, as revealed in experimental anaphylaxis in animals and in asthma in man.

Substances of high molecular weight as well as intact cellular structures, such as bacteria or blood cells, have been found to be antigens. A variety of methods for the demonstration of antibodies against these antigens has been developed, and the list is constantly increasing. The precipitation of soluble antigens has been most thoroughly studied. This technique allows the demonstration of small amounts of antigenic material, sometimes in dilutions up to several millions. No known chemical reagent allows the recognition of the presence of protein in such minute quantities.

In the 1940s investigations of Oudin and Ouchterlony opened new vistas for demonstrating multiple antigens in biologic mixtures that formerly were considered to contain only one or two antigenic constituents. By carrying out the antigen-antibody reaction in a gelified medium, such as agar, bands of precipitation form wherever an antibody and its corresponding

antigen meet at the optimal proportion. The procedure of immunoelectrophoresis elaborated by Grabar and Williams combines electrophoretic separation of the antigen with agar-gel precipitation. This is an exquisite procedure for detection and characterization of antigens in an antigenic mixture (see Chapter 4). By means of immunoelectrophoresis over 30 antigens could readily be identified in blood plasma.

Antigens occurring on intact cells, such as bacteria and blood cells, are easily demonstrated by agglutination, by lysis in the presence of complement, or by phagocytosis in the presence of phagocytic cells. These techniques, too, are undergoing intensive study, and new observations are improving our understanding of the interaction of antibodies and antigens present in cellular structures. There remain, however, a number of antibody functions recognized best by neutralization. Neutralization tests are used, for instance, for the recognition of the presence of antibodies against virus particles. Of great interest is the study of cytotoxic effects of antibodies combined with their corresponding antigens on the living cell. In addition, the localization of antigens in tissues can be pinpointed by use of the antibodies labeled with radioactive isotopes, fluorescent compounds, or enzymes (see Chapters 8 and 9).

Antibodies and Their Properties

The different forms of antibodies recognized were named according to their observable effects—*precipitins* when soluble antigens were dealt with, *agglutinins* and *lysins* when cells were involved, and *opsonins* in connection with phagocytosis. However, it was long ago realized that these different antibodies could not be readily distinguished from each other. An antiserum produced in rabbits by injecting extracts of typhoid bacilli would also agglutinate intact whole typhoid bacilli. Conversely, an antiserum produced by injection of whole typhoid bacilli would precipitate an extract of typhoid bacilli. For this reason a unitarian concept was developed according to which an antibody function recognizable in the test tube would depend on secondary experimental conditions rather than on differences in the antibody molecule. This unitarian concept should be considered a valid rule even though numerous exceptions could be quoted.

One of the most interesting and most useful characteristics of an antigen-antibody reaction is its specificity. An antitoxin produced by injection of diphtheria toxin will neutralize only diphtheria toxin but not the toxin produced by any other kind of bacterium, and an agglutinin produced against typhoid bacilli will clump typhoid bacilli but not other unrelated microorganisms. Thus, the living animal body, by means of antibody formation, manufactures reagents that allow the identification of microbial strains isolated from patients, supplementing the cultural and biochemical

methods of characterization. In many instances the immunologic tools permit the classification of bacterial species into various subtypes that differ from each other exclusively by their antigenic composition. The specific antiserum serves also as a remarkable reagent for the detection of specific constituents in bacterial and tissue cells, and it serves as a guide in their isolation, purification, and final chemical analysis (see Chapters 24 and 29).

Upon fractionation of an antiserum, antibody activity is found in the γ- and β-globulin fractions. The globulins carrying antibody activity are called immunoglobulins. There are at least five classes of immunoglobulins: IgG, IgM, IgA, IgD, and IgE. Antibodies of the IgG class are the most frequent. They have a sedimentation rate of 6 to 7 S. IgM antibodies are often the first antibodies to appear during immunization. They are approximately six times larger than IgG antibodies and their sedimentation rate is around 19 S. The IgA antibodies occur as monomers with a sedimentation rate of 7 S, as dimers with a sedimentation rate of 10 to 11 S, and even, in small amounts, as trimers and tetramers (13 S and 16 to 17 S). IgE has the unique ability to attach avidly to cells of the same species and to mediate hypersensitivity reactions. The immunoglobulins are discussed in Chapter 10.

Antibodies are formed by lymphoid tissues, mostly in lymph nodes and in the spleen. The cells mainly involved in the production of antibodies are lymphocytes and plasma cells. Two types of lymphocytes seem necessary to initiate antibody formation, a thymus-dependent and a bone marrow–derived (or, in birds, bursa-dependent) lymphocyte. Macrophages may also be involved. The cellular interactions, however, have not yet been entirely worked out (see Chapter 15).

Many theories have been proposed to account for antibody formation (see Chapter 25). Instructive theories imply that the antigen itself is directly or indirectly involved in the formation of a template by means of which the cell manufactures immunoglobulins with a structure complementary to the antigen. Selective theories are based on the assumption of the preexistence of all possible antibody patterns. The invading antigen selects the complementary pattern and increases its production. The clonal selection theory of Burnet proposed that cells involved in formation of a corresponding antibody are stimulated by the antigen to multiply, which gives rise to a clone of cells forming the particular antibody. This ingenious theory inspired many valuable investigations and underlies much of the contemporary research in cellular immunology.

Specificity of the Immune Response

The outstanding specificity of antigen-antibody reactions is not absolute, and cross-reacting antigens are quite frequently encountered. From the biologic standpoint it was understandable that antigens of related species,

such as typhoid and paratyphoid bacilli, or, as far as animal protein is concerned, serum proteins of man and those of anthropoid apes have similar molecular structures that would be responsible for the cross-reactions exhibited by them. However, it was very difficult at first to interpret the discovery of the Swedish pathologist Forssman. Forssman injected suspensions of guinea pig kidney into rabbits. The antiserum obtained would not only precipitate guinea pig protein but it also contained sheep cell hemolysins of high titer. Furthermore, antisera against organ suspensions of other animals, such as horses, cats, and certain birds and fish, would also contain sheep cell hemolysins. To the surprise of everybody concerned, it could be shown that indeed a very similar chemical structure is present in the cells and tissues of these entirely unrelated species.

Forssman antibodies, demonstrable by their action on sheep cells, can be removed by extracts or suspensions of any of the cells and tissues that contain the Forssman antigen. Indeed, the entire animal kingdom can be divided into two groups: animals that contain the Forssman antigen, referred to as the guinea pig type; and animals such as rabbits, cattle, and pigs that do not contain the Forssman antigen and are referred to as the rabbit type. It was quite a revelation for the immunologist to learn that the Forssman antigen is soluble in 80% ethanol. At the time of the discovery of the ethanol solubility of the Forssman antigen only true proteins were believed to be antigens. However, another surprise was in store for the investigators. Basically, an antigen has two outstanding characteristics—it induces antibody formation in the living animal, and it reacts with the corresponding antiserum in the test tube. However, ethanol extracts of organs containing the Forssman antigen act like legitimate antigens in the test tube, but fail to elicit antibody production upon injection into rabbits. In the case of the Forssman antigen, the dual characteristics of an antigen become apparent. The Forssman antigen in its natural state is always connected with proteins foreign to the rabbit, the antibody-producing animal. By the procedure of ethanol extraction a lipid is separated from the protein, and this lipid by itself fails to stimulate antibody production but still acts as an antigen in the test tube. Finally, Landsteiner showed the significance of the two components by mixing Forssman lipid with a foreign protein, such as pig serum protein. This mixture, when injected intravenously into rabbits, stimulated antibody formation against both constituents, namely, Forssman antigen as well as pig serum protein. The term "hapten" was then coined by Landsteiner to denote a substance that, although acting as an antigen in the test tube, must be combined with some foreign protein in order to induce antibody production *in vivo*.

Studies on the Forssman antigen profoundly shattered the original belief of the immunologist that only proteins were endowed with antigenic properties. Soon other nonprotein antigens were described. The type specificity of pneumococci was found to be due to water-soluble substances present in the capsule of virulent pneumococci. Avery and Heidelberger identified these

substances as complex carbohydrates that act as bearers of this type-specific differentiation of pneumococci. When purified, these type-specific carbohydrates are protein-free. They are readily precipitated in the test tube by the addition of type-specific antisera, i.e., antisera obtained by immunization with encapsulated, intact pneumococci. Yet these isolated complex carbohydrates fail to elicit antibody production when injected into the rabbit and, therefore, appear to be haptens. Following the experiments of Landsteiner, who succeeded in activating the Forssman hapten to full antigenic capacity by simply mixing it with pig serum, a similar procedure was applied to the pneumococcal carbohydrates but failed in this case. Actually only by direct chemical combination with a foreign protein, as by diazotization, can complex carbohydrates be rendered antigenic for a rabbit. Even simple chemical substances of known configuration can be coupled to proteins and, following injection into rabbits, they stimulate antibody formation, acting as full antigens. Studies of hapten-protein conjugates have done much to elucidate the basis of immunologic specificity (see Chapter 11).

The pneumococcal polysaccharides generally do not contain nitrogen. Heidelberger and Kendall, therefore, hit upon the idea of precipitating all of the antibody from an antiserum with a slight excess of specific polysaccharide, carefully washing the precipitate and measuring its content of nitrogen, which must be attributed to antibody. Actually, the same procedure can even be used for determining the amount of antibody reacting with protein antigen if the nitrogen contributed to the precipitate by the antigen is allowed for by appropriate calculations. The method of antibody nitrogen determination as introduced by Heidelberger and coworkers ties chemical precision to immunologic reactions by giving the immunologist quantitative methods for measuring the amount of precipitating antibody present in antiserum (see Chapter 4).

Phylogeny of the Immune Response

Even in his earliest works, Metchnikoff realized that immunologic competence—the ability to recognize the endless array of extrinsic substances—is a property of all cells. Its primary adaptive function is to maintain the integrity of the body and to recognize food. In protozoa, for instance, phagocytosis serves needs of both nutrition and defense. In metazoa these functions separated, the endoderm subserving the main nutritional needs while the mesoderm developed special mechanisms of defense. There is evidence that invertebrates (such as earthworms) recognize and reject skin grafts of other species and that the hemolymph of crustacea and insects reacts specifically with certain antigens. The question of whether such animals develop an anamnestic response to a second exposure to the same antigen requires further attention.

It is among vertebrates that the immune response seems to have evolved in its most elaborate form. In the relatively primitive Ostracodermi like the hagfish and lamprey, which evolved 480 million years ago during the Ordovician period, one finds the ability to reject incompatible grafts in an accelerated or "second-set" manner and to produce specifically reactive immunoglobulins in response to injection of foreign erythrocytes. Even the general structure of their immunoglobulins is comparable to that of mammals, comprising associated light chains and heavy chains. Although they may or may not have the precursor of the thymus, these animals have families of circulating lymphocytes, and lymphopoiesis can sometimes be seen in the spleen. In elasmobranchs, a thymus can be defined—it even involutes with age—and plasma cells are recognized. Holostean and teleostean fish show at least two distinct classes of immunoglobulins with an IgM to IgG conversion following immunization.

The immune response in birds is more clearly dichotomous than in other classes. The thymus is necessary for the development during early life of the cell-mediated functions such as rejection of grafts, and a lymphoid organ near the cloaca, the bursa of Fabricius, is required for development of circulating antibodies. The interaction of thymus-dependent (T) lymphocytes and bursa-dependent (B) lymphocytes can be studied to great advantage in birds.

The evolutionary conservatism of the immunologic system, including many details of immunoglobulin structure, emphasizes its importance in survival. Immunology, therefore, has expanded from a specialty of medicine to the natural science concerned with recognition, surveillance, and maintenance of integrity of the individual organism.

Bibliography

Bolduan, C., and Ehrlich, P. (eds.): *Studies in Immunity*. John Wiley & Sons, New York, 1910.

Bordet, J.: *Traite de l'Immunite dans les Maladies Insectieuses*. Masson et Cie, Paris, 1939.

Boyd, W.C.: *Fundamentals of Immunology*. Interscience Publishers, New York, 1966.

Coombs, R. R. A., and Gell, P. G. H. (eds.): *Clinical Aspects of Immunology*. F. A. Davis, Co., Philadelphia, 1968.

Haurowitz, F.: *Immunochemistry and the Biosynthesis of Antibodies*. Interscience Publishers, New York, 1968.

Heidelberger, M.: *Lectures in Immunochemistry*. Academic Press, Inc., New York, 1956.

Kabat, E. A.: *Structural Concepts in Immunology and Immunochemistry*. Holt, Rinehart & Winston, Inc., New York, 1968.

Landsteiner, K.: *The Specificity of Serological Reactions*. Dover Publications, Inc., New York, 1962.

Long, E. R.: *A History of Pathology*. Dover Publications, Inc., New York, 1965.