basic & clinical immunology

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Preface

The past three decades have been marked by rapid advances in all medical and technologic fields pertaining to immunology, so that now this branch of medical science clearly ranks as a special discipline in its own right rather than a subdivision of microbiology or allergy.

With all this interest and activity has come inevitable expansion in the variety and bulk of the supporting literature. There are now a multitude of journals primarily devoted to immunologic topics, and almost all of the medical journals are soliciting or accepting articles on the immunologic features of disease, organ transplants, basic and applied research in immunology, tumor immunology, and immunotherapy.

There are many good books on the subject, and we hope we have not neglected to list any of them in the bibliography pages at the ends of the chapters. Observing that events seemed to have outrun our best competition, the editors decided that another book was justified. *Basic & Clinical Immunology* is already written as we prepare this Preface, and we note that the men and women who make the events happen are still not idle. The result is that as we examine page proof and prepare the Index we are already working on the Second Edition, due in 1978, and will continue to offer new editions biennially. *For these future publications, the reader's comments are urgently solicited.*

The book is divided into four sections, and the sequence of chapters is intended to be logical. The first section describes the fundamentals of immunochemistry and cellular immunology. The second section applies this knowledge to a discussion of immunobiology, an area that bridges basic and clinical immunology. The third section describes the immunologic laboratory tests that are available for evaluation of patients. The fourth section presents the immunologic aspects of a variety of human diseases.

The clinical chapters focus on primary immunologic diseases or on disorders with important immunopathologic characteristics. These discussions are not intended to serve as a manual of clinical treatment; where specific medications or drug dosages are mentioned, the physician should also consult more comprehensive medical texts.

It is hoped that this book will serve as a text for medical students, house officers, graduate students, practicing physicians, and others interested in learning more about the field. Immunologists from both basic and clinical disciplines should find it a comprehensive review.

The support provided by the staff of the publisher—particularly Drs Ransom and Lange—has been invaluable in this venture, and they have our deep appreciation. Much of the art work was done by the expert hand of Ms Laurel Schaubert, who has our thanks. Finally, we wish to express our gratitude to the contributing authors whose knowledge it is that represents whatever value this book may have; and to the institutions that have supported us during the long task of preparing this book.

—The Editors

San Francisco
September 1976

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Section I

Basic Immunology

1...

The Historical Background of Immunology

Pierre Grabar, DSc

Immunology is a relatively young branch of medical science. Many observations of importance to immunology were made by microbiologists around the turn of this century, usually in the course of active research in bacteriology and infectious diseases. For many years immunology was studied as part of microbiology, and progress in the field consisted mainly of application of what had been learned about immunologic phenomena to the problems of the diagnosis and control of bacterial infections. Some of the most important advances were made possible by the introduction of chemical technics in the elucidation of the nature of antigens and antibodies.

The explosive increase in fundamental information has made immunology an independent branch of science. *Zeitschrift für Immunitätsforschung* began publication in 1909 and the *Journal of Immunology* in 1916. There are now 25 national member societies in the International Union of Immunological Societies. This chapter will outline some of the contributions by pioneers in immunology which have led to the current state of the art. Where appropriate, reference is made to relevant chapters in this book.

The term *immune* derives from Latin *immunis*, ie, exempt from "charges" (taxes, expenses). However, for nearly a century the term immunity has denoted resistance to possible attack by an infectious agent. Resistance to second attacks of certain diseases had been observed even in ancient times. Attempts to protect against variola (smallpox) were made in ancient China and western Asia by inoculation (variolation) using vesicle fluid from persons with mild forms of smallpox, or by purposely seeking out contact with diseased individuals. Lady Mary Wortley Montagu (1721) introduced into England from Turkey the process of *variolation*, or inoculation with unmodified smallpox virus. It was quite dangerous since disease and death often resulted. Similarly, an ancient Greek king of Pontus, Mithridates VI, tried to protect himself against the effects of poison by administering small amounts of poisonous substances on multiple occasions—a procedure that came to be called *mithridatism*.

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EARLY IMMUNOLOGY

The first effective—though still empirical—immunization was performed by Edward Jenner, an English physician (1749–1823), who observed that persons who got well after infection with cowpox were protected against smallpox. Jenner introduced vaccination with cowpox in 1796 as a means of protecting against smallpox. The term *vaccination* (L *vacca* cow) was introduced to replace the term variolation.

The scientific approach was not applied to the study of immunologic phenomena until almost a century later as a consequence of work on microbes by Louis Pasteur (1822–1895) and his collaborators. They investigated the possibility of protecting against infection by vaccinations with attenuated strains of microorganisms. Their first observation (1878–1880) was that a culture of *Pasteurella aviseptica* (then called chicken cholera) which had been left in the laboratory during vacation lost its virulence for chickens, and that animals inoculated with this culture were protected against the virulent strain. Pasteur concluded that this culture contained attenuated microbes and, to honor the work of Jenner (nearly 100 years before), extended the term vaccination to denote conferring immunity by injection of attenuated strains of organisms. The idea of using attenuated strains of microorganisms was confirmed by Pasteur when he studied vaccination against anthrax (1881). Research on the mechanisms of protective effects led Richet and Héricourt to the conclusion (1888) that the blood of an animal immunized with staphylococci conferred partial protection against subsequent inoculation with these microorganisms. The next year, Charrin and Roger observed that the serum of an animal immunized with *Pseudomonas aeruginosa* (then called *Bacterium aeruginosum* among other names) agglutinated a suspension of this microbe.

In 1889, Pfeiffer, a pupil of Koch, used cross-immunization of guinea pigs with 2 similar microbes

(*Vibrio cholerae* and *V metchnikovii*) to show that it was possible to distinguish them immunologically, since immunization against one did not protect against the other. The specificity of the protective effects of immunization had already been observed, but this example showed how extremely fine the specificity could be in some cases (Chapters 2 and 3).

"CELLULAR IMMUNITY" THEORY

In 1882 in Messina the Russian zoologist Elie Metchnikoff (1845–1916) studied the role of motile cells of a transparent starfish larva in protection against foreign intruders. He introduced a rose thorn into these larvae and noted that a few hours later the thorn was surrounded by motile cells. This experiment can be considered the starting point of cellular immunology. It had already been established by Koch and Neisser that bacteria can be found in leukocytes, but it was thought that this was the result of bacterial invasion of the leukocytes. Metchnikoff showed that the leukocytes had in fact engulfed the microorganisms. In 1883, Metchnikoff observed that Daphnia, a tiny transparent metazoan animal, can be killed by spores of the fungus *Monospora bicuspidata* and that in some instances these spores are attacked by blood cells and can be destroyed in these cells, thereby protecting the animal against the invaders. In 1884, he extended these observations to the leukocytes of rabbits and humans, using various bacteria. He noted that the engulfment of microorganisms by leukocytes, which he called *phagocytosis*, is greatly enhanced in animals recovering from an infection or after vaccination with a preparation of these microorganisms. He therefore concluded that phagocytosis was the main defense mechanism of an organism. He later showed the existence of 2 types of circulating cells capable of phagocytosis—the polymorphonuclear leukocytes and the macrophages—as well as certain fixed cells capable of phagocytosis, and proposed the general term *phagocytes* for all of these cells (Chapter 7).

The *cellular immunity* theory of Metchnikoff, who worked at the Pasteur Institute in Paris from 1887, was accepted with enthusiasm by some but was criticized by several other pathologists. The inflammatory reaction had been described by Celsus as early as the first century AD, but before Metchnikoff it had been studied only in mammals. Pathologists such as Virchow (1871) agreed that inflammation was due to changes in the connective tissue cells induced by various agents, particularly by abnormal deposits of metabolic products. Cohnheim (1873) and his collaborator Arnold (1875) considered inflammation to be a local vascular lesion due to a noxious agent which allowed blood cells to penetrate into tissues. Metchnikoff, who had observed the same accumulation of motile cells in lower animals with no circulatory vessels, asserted that diapedesis in higher animals was a process of active penetration of these cells through the walls of the vessels (1892). In his opinion, inflammation resulted in an enzymatic digestion process due to ingestion of the noxious agent by the motile phagocytes.

Metchnikoff's theory came under severe criticism somewhat later by those who observed immunity in the absence of cells. Fodor in 1886 was apparently the first to observe a direct action of an immune serum on microbes during the course of his studies on anthrax bacilli. Behring* and Kitasato (1890) demonstrated the neutralizing antitoxic activity of sera from animals immunized with diphtheria or tetanus toxin, which was considered the first proof of humoral immunity. In 1894, Calmette observed the same neutralizing activity of snake venom antiserum. The preparation of large amounts of diphtheria toxin antiserum in horses for human use started almost immediately in the Behring Institute in Marburg (Germany) in 1893; at the Pasteur Institute in Paris (Roux and Martin, 1894); in the USA; and also at the Lister Institute in London (1895). This was the beginning of serum therapy, a form of treatment which developed remarkably over the next 50 years.

"HUMORAL" THEORY

An important humoral defense mechanism described by Pfeiffer and Isaeff (1894) has come to be called the *Pfeiffer phenomenon.* Cholera vibrios injected into the peritoneum of previously immunized guinea pigs lose mobility, are clumped, are no longer stainable, and are later phagocytosed by leukocytes, but they are also lysed in the absence of cells.

A theory of immunity due to humoral factors provoked intense debate between Metchnikoff and the supporters of this new theory, mainly from the laboratory of Robert Koch (1843–1910). At the time of Pfeiffer's discovery, a young Belgian, Jules Bordet (1870–1961), was engaged in the study of agglutination reactions in Metchnikoff's laboratory at the Pasteur Institute. He became interested in the Pfeiffer phenomenon and in 1895 showed that both bacteriolysis and lysis of red cells (which he described in 1898) required 2 factors: one, which he called *sensitizer*, was thermostable and specific; the other, which he called *alexine*, was thermolabile and nonspecific. The factor designated alexine by Bordet came to be called *cytase* by Metchnikoff and *complement* by Ehrlich (Chapter 6). Bordet believed that his "alexine" possessed enzymatic activity and that it consisted of several components.

It is of interest that Bordet's studies of humoral factors were performed in Metchnikoff's laboratory and were in contradiction to the master's theories. Later, both theories gained general acceptance and it

*The particle von was added later to Behring's name after he became famous—about the time he received the Nobel Prize.

was established that humoral factors originated from lymphoid cells.

During this period, the term *antigen* was introduced to designate any substance (then mainly microbes or cells) capable of inducing a reaction against itself and the illogical term *antibody* (both being "anti-") to designate the factor present in the serum possessing this activity. At first, various special names were used to indicate each observed antibody activity, such as *agglutinins, precipitins, sensitizers,* and *opsonins* (Chapters 4 and 16). The first observation of agglutination is described above. The precipitin reaction was described later—in 1897 by Kraus with microbial culture supernates and the serum of immunized animals, and in 1899 by Tchistovitch with serum protein antigens and by Bordet with milk antigens and serum of animals injected with these fluids. The precipitin reaction was introduced by Wassermann and Uhlenhuth into forensic medicine for the identification of blood or meat.

Complement & Immunologic Diagnosis

The term *sensitizer* was used by Bordet to denote the thermostable serum component reacting in the lysis of bacteria. Ehrlich called it *amboceptor.* In 1900, Bordet established the reaction which he designated *alexine (complement) fixation or deviation,* using cholera vibrios, a corresponding immune serum, and complement (fresh normal serum). As a control of fixation of the latter, he added red cells sensitized by the homologous antiserum; the absence of hemolysis proved that the complement had been absorbed by the first reaction (Chapter 6). This system allowed him to make quantitative estimations. In 1901, he described (with Gengou) this general principle, which is still valid and useful and which was first applied by Wassermann, Neisser, and Bruck in 1906 in the diagnosis of syphilis using tissues from syphilitic patients as antigen. Various modifications of this method have been subsequently introduced, eg, the use of an alcoholic extract of guinea pig heart (reagin) as antigen (Landsteiner) and, particularly, the use of lipids (see Chapter 34).

The classical method of diagnosis of typhoid fever by salmonella agglutination reactions was established independently in 1896 by Widal in France and by Durham. The latter worked at that time in Germany in Gruber's laboratory, where agglutination reactions had been under study for several years. A method of diagnosing pneumonia proposed in 1902 by Neufeld consisted of observing the swelling *(Quellung)* of the capsules of pneumococci when the microorganisms were exposed to patient serum containing antipneumococcal antibodies (see Chapter 34).

Resolution of Conflicting Theories

In 1895, Denys and Leclef observed the fixation of antibodies present in an antistreptococcus serum by these organisms and called them *bacteriotropins.* Neufeld and Rimpau had also demonstrated similar in vitro fixation. In 1903, Wright and Douglas, after a careful study of Metchnikoff's observation that phagocytosis of microbes is facilitated by the serum of an immunized animal, used washed cells to demonstrate that the immune serum contained an active factor they called *opsonin.* They proposed the term *opsonization* for the activity, and this phenomenon acted as a "bridge" between the apparently contradictory humoral and cellular theories.

During this same period, Paul Ehrlich (1854–1915) studied the neutralization of toxins by immune serum, using the highly toxic vegetable poisons abrin and ricin, which could be extracted easily in sufficient quantity. These studies enabled him to establish a technic for the evaluation of the antitoxic activity of diphtheria antiserum (1897).

EHRLICH'S "SIDE-CHAIN" THEORY

Ehrlich was interested in the theoretic aspects of immunologic phenomena and in 1896 elaborated his *side-chain theory* to explain the appearance of antibodies in the circulation. He considered it an "enhancement" of a normal mechanism and suggested that cells capable of forming antibodies possessed on their surface membrane specific side chains which were receptors for antigens. He proposed that binding of antigen to the side chains provoked new synthesis of these side chains, which were liberated into serum as antibodies. He expressed the specificity of the reaction of antigens and antibodies as a "key [antigen] in a lock [antibody]" and thought that this reaction was of a chemical nature. During the next few years, he tried to substantiate his theory with various arguments, but the theory was not generally accepted. It was criticized by Bordet, who felt that the antigen-antibody reaction was of colloid nature; by Gruber; and particularly by Arrhenius and Madsen, who insisted on the reversibility of the reaction and on different proportions of reactants in specific precipitates. Nevertheless, Ehrlich's general theory, with modifications and additions, has been taken into consideration by many authors, and his hypothesis on the existence of specific receptors on immunocompetent cells has recently been completely vindicated (Chapter 8).

The transmission of antibodies across the placenta or through the mammary gland in milk from maternal mice to their offspring was observed by Ehrlich in 1892. In 1893, Klemperer demonstrated the existence of antibodies in the eggs of poultry and therefore transmission of antibodies to newborn chicks.

Isoantibody

In 1875, L. Landois published his monograph *Blood Transfusion.* He noted the effects of blood transfusions between members of different species and observed it was preferable to work within a single species. He also stated, however, that there were differences within a single species since a recipient's own cells could be hemolyzed by serum from a nonidentical donor of the same species.