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Page 1
in 1877
1877

PROGRESS IN ALLERGY

FORTSCHRITTE DER ALLERGIELEHRE

Vol. VI

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With 78 figures and 38 tables

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BASEL (Switzerland)

S. KARGER

NEW YORK

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Printed in Switzerland by Buchdruckerei Aargauer Tagblatt AG, Aarau.
Clichés: Steiner & Cie. AG, Basel.

Preface

The excellent work of the contributors and the publisher have secured the "Progress in Allergy" series a place on the bookshelves of many libraries and on the desks of immunologists and allergists all over the world.

Owing to the considerable expansion made in the field of allergy during the last few years, it will be necessary to publish "Progress in Allergy" more often in the future, and I therefore have great pleasure in announcing that Dr. BYRON H. WAKSMAN of Boston has kindly agreed to share the editorship with me. The present volume is the first result of our joint editorship and I should like to express my warm appreciation of Dr. WAKSMAN's co-operation.

P. KALLÓS

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Introduction

By P. KALLÓS AND B. H. WAKSMAN

In a spirited and inspiring lecture on "Episodes in Immunochemistry" M. HEIDELBERGER (11) states that a method "that gained rapid acceptance was the mutual diffusion of antigen and antibody in gels, first made practical by OUDIN in 1946 and greatly extended in its possibilities by the substitution of agar plates for tubes by OUCHTERLONY. The fascinating pattern of lines so obtained often permit conclusions as to the number of antigens in a system, their molecular weights, and, some times, with the help of proper staining, the presence or absence of components, such as carbohydrates, lipids, or nucleic acid." The great usefulness and growing importance of OUCHTERLONY's technique also in combination with electrophoresis ("immunoelectrophoresis") is mirrored in the number of publications on its use in all special fields of biology and medicine. Since the thorough review by OUCHTERLONY in the previous volume of our "Progress in Allergy" several thousands of papers have been published in this field. It is certainly a great advantage that OUCHTERLONY continues his review in the present volume (p. 30). The structure of antigens and antibodies and the mechanisms of their interaction are still in the center of interest and gel-diffusion and immunoelectrophoresis are certainly very important tools for instance in the comparison of various antigens and antibodies, in the identification of them in undefined mixtures and in revealing cross reactions. OUCHTERLONY points out the advantages and the pitfalls of the different techniques and gives many examples for their application. He also provides a selected bibliography, covering the most important publications on pertinent problems.

It is a most important event that the whole field of immunochemical methodology has been thoroughly reviewed in the long awaited and entirely revised second edition of the classical monograph "Experimental Immunochemistry" by E. A. KABAT AND

M. M. MAYER (52). As KABAT stresses in his preface "recent immunochemical advances have provided an approach to the elucidation of the structure of antigenic groupings, especially the polysaccharides, so that one can already envisage a period of intense activity along these lines. . . . These efforts have simultaneously provided information both on the size and heterogeneity of antibody combining sites. In addition degradation studies with enzymes have shown that fragments containing antibody combining sites and various antigenic determinant groups may also be obtained. One hopes that the analytical and the degradative approaches will ultimately meet to give a more complete knowledge of the structure of antigenic groups and of antibody combining sites." All contributions to the present volume are related to the problems, outlined by KABAT.

An interesting practical example for the usefulness of the immunoelectrophoretic technique is the discovery of a new genetically determined serum protein system (Gc) in normal human sera by J. HIRSCHFELD. We feel that this thorough work has a given place in this volume (p. 155) not only from the methodological point of view but also as a new and conclusive evidence for the existence of genetically determined serum protein differences between individuals of the same species. The two group specific (Gc) components occur in the α_2 -globulin region and are electrophoretically different but interestingly enough immunologically indistinguishable. The important problems of protein structure and metabolism were the subject of two recent symposia (18, 19).

In his introduction to the previous "Progress"-volume P. KALLÓS discussed the different theories of antibody production, the instructive *vs.* the elective hypothesis. At that time the latter was represented by JERNE's "natural selection theory" (further elaborated by him in 49), which postulates that among the gammaglobulin molecules, which are produced by the organism in an "enormous variety of different configurations", there will exist molecules, the surface pattern of which is by chance complementary "to any antigen to which the animal can respond." If such natural antibodies are available at the moment when an antigen enters the circulation, they will combine with the antigen. Then, the antigen-antibody complex is rapidly removed from the circulation by the phagocytic and lymphatic system and reaches the plasma cells, the site of globulin synthesis. The plasma cells then tend to selectively

and preferentially synthesize gammaglobulin molecules "identical to those introduced, i. e. specific antibodies." As pointed out by KALLÓS, the natural selection theory has many attractive features, it is however, not able to explain all different aspects of antibody production satisfactorily.

In 1958 Sir MACFARLANE BURNET (13, 14) launched his "clonal selection theory" of acquired immunity, a development of JERNE's hypothesis. BURNET stresses that the protein pattern is genetically determined. Protein producing cells and their descendants own in their genetic mechanism appropriately coded information. Somatic mutation of the cell can modify the pattern of the protein, produced by the cell. "It is universally accepted" according to BURNET "that the phenomena of immunity are based on the functional activity of populations of mesenchymal cells within the body", thus "immunological specificity is based on the special type of differentiation occurring in embryonic life plus a high subsequent potential for somatic mutation in that region of the genome (using this term in the broadest sense to cover all genetic determinants, nuclear and cytoplasmic) concerned with immunologically significant pattern." Accordingly, the type of γ -globulin produced by plasma cells is determined by the genetic character of the clones of cells concerned. The mobile population of cells, capable of antibody production, "carry surface sites analogous to the specific pattern of the antibody globulin they produce." The corresponding antigenic determinant "selects" such a cell and stimulates a proliferative response "which allows a selective advantage to the clone concerned." Moreover, BURNET postulates that in the course of embryonic development "an active process by which all clones that carry active sites corresponding to antigenic determinants present in the accessible parts of the body are eliminated" takes place. This would explain "immunological tolerance" and the inability of the immunologically mature organism to produce antibodies directed towards its own accessible antigens ("self"). The influence of an antigen during embryonic life (and the very first days after birth) causes according to this theory elimination of the corresponding clone of antibody producing cells, whereas contact with an antigen after this "critical point is assumed to stimulate the cell to functional activity." This unexplained difference is perhaps one of the weak points of this very stimulating theory. In the few years after BURNET's presentation of the clonal selection theory a great number of papers has been pub-

lished on BURNET's theory and several modifications have been proposed (8, 11, 14, 20, 28, 35, 43, 45, 49, 62, 63, 74, 100, 102, 103, 106a, b, 108, 109, 110, 111, 121, 126, 127, 128).

Some of the modifications should perhaps be specifically mentioned. J. LEDERBERG (63) stresses the central role of microsomes in protein synthesis. According to him "a powerful elective theory is generated by substituting the term *microsomal RNA* for the term *chromosomal DNA* and *gen.*" "Since a single cell may have millions of microsomes, this theory would allow for any imaginable multiplicity of antibody forming information in a single cell." This assumption places selectivity on a subcellular level and this gives the hypothesis greater flexibility. In two recent papers L. SZILARD deals with the control of the formation of specific proteins in bacteria and in animal cells (106a) and with the molecular basis of antibody formation (106b) respectively. The starting point of his discussion is the phenomenon of enzyme repression and its importance for the production of so-called adaptive enzymes. He provides a thought-provoking theory of antibody synthesis, which certainly will influence future experimental work. Independently of him WEISSMAN AND LUSTGRAF (121) recently proposed that repressor systems, similar to them which govern enzyme synthesis in microorganisms, may operate in antibody formation. In his opening remarks to a panel discussion on "Biosynthesis of Antigens and Antibodies" at a recent symposium (43) H. J. VOGEL stated that if "one favors elective theories of antibody formation, a repression type mechanism would seem recommend itself." He refers to his work on enzyme repression and to "a unified hypothesis of repression and induction, the regulator hypothesis, which contemplates, *inter alia*, that the induction of an enzyme may represent a counter action to a repression." It seems quite possible that much future work will be done along these lines.

There seems to be agreement that the immunological competent antibody producing cell is the plasma cell (126, 37, 39, 43, 45, 60, 62, 64, 71, 82, 88, 100, 107, 108). We refer to the discussion of the pertinent problems by R. A. GOOD et al. and by M. SIMONSEN in this volume. The role of the precursors of the plasma cell in the quite heterogenous cell population of the lymphoid tissue system, lung, spleen and bone marrow, is less clear.

At a recent symposium on "Immunochemical Approaches to Problems in Microbiology" (43) NOSSAL AND MÄCKELÄ analyzed

the inhibition and restoration of the specific antibody response in a very stimulating way. The antibody response can be inhibited in different ways. The exposure of animals to an antigen before immunological maturation (i. e. before or immediately after birth) causes a specific inhibition of antibody production during later life ("immunological tolerance"). Introduction of isologous immunologically competent cells (spleen or lymph node cells) terminates the tolerance, showing that specifically reactive cells were lacking in the tolerant animal. Very large doses of certain antigens (for instance pneumococcal polysaccharides or foreign proteins) render adult animals specifically unresponsive ("immunological paralysis"). It is quite possible that the cellular mechanism in this later state is similar to that in the tolerant state. Lethal or nearly lethal doses of X-rays cause lymphoid necrosis and inhibit the antibody response thereby. If the dose is sublethal compensatory lymphoid proliferation takes place and responsiveness will be restored. After a lethal dose implantation of isologous, homologous or heterologous immunologically competent cells can restore immunological responsiveness and save the animal. NOSSAL AND MÄCKELÄ point out that some antigens, such as serum proteins and foreign erythrocytes can easily induce tolerance, others, for instance pneumococcal polysaccharides, cause easily immunological paralysis, whereas gram negative bacterial antigens, which are truly foreign to the animal organism, are not capable of inducing either state of immunological unresponsiveness. The bacterial antigens are "good" antigens, they cause a "brisk plasma cell response and a good serum antibody formation on first injection", which is not easily depressable by steroids or antimetabolites. Serum proteins, erythrocytes and polysaccharides are "poorer" antigens, plasma cell proliferation and antibody formation on first injection are not so impressive. Similar observations have also been reported by MITCHISON (74). NOSSAL AND MÄCKELÄ showed furthermore that the exposure of mice "following lethal irradiation and implantation of isologous spleen cells" during the early stages of the recovery period, when the majority of the immunologically responsive cells must be regarded as immature, to large amounts of rat erythrocytes, did in none of a variety of experimental situations induce even partial tolerance. In another set of experiments NOSSAL AND MÄCKELÄ irradiated rats "in which a state of immunological tolerance to mouse erythrocytes had been induced." "Surprisingly, when tolerant animals were lethally irra-

diated and saved with a homograft of spleen and bone marrow taken from another tolerant rat, these tolerant-tolerant chimeras began to make quite high titers of antibody." NOSSAL AND MÄKELÄ assume that "during embryonic life, mesenchymal cells differentiate into clones of differing immunological potential." "Natural selection might favor the development of more clones of useful character, if this is chemically possible" and "very small doses of antigens reaching the fetus from the mother's circulation may be stimulatory even in the embryo." These two factors would explain that bacterial antigens are "good"; the antibody response to them is possibly never truly primary. NOSSAL AND MÄKELÄ postulate that the immunologically competent cells within a clone are in different functional states: "some active multiplying, or immediately sensitive to any stimulans; some resting; some possibly in intermediate states. The overall trend in the unstimulated animal may be more and more towards the resting state." Antibody formation goes on stepwise. *Step 1*: the sensitive cells, possibly large lymphocytes multiply. *Step 2*: a number of the cells so stimulated differentiates into plasma cells "with limited further multiplication on the way." *Step 3*: another number of the stimulated large lymphocytes differentiates to resting or memory cells, possibly small lymphocytes. *Step 4*: The antigen stimulates the memory cells too and these are capable of re-entering the cycle as "primitive competent cells, possibly large lymphocytes." All the above mentioned experimental results can be satisfactorily explained with the help of some further assumption: Firstly, "that steps 3 and 4, and possibly step 1, are inhibited by high concentrations of antigen but step 2 is not." Secondly, "that there are cellular interactions which ensure that a clone with many cells always has a reasonable number of cells in the sensitive state. Adult antigenic stimuli of course create more sensitive cells." Thirdly, "that the cellular mechanisms in the embryo are basically similar but set at lower thresholds of antigen; relatively low concentrations of antigen will block steps 3 and 4; step 2 may be impossible in the embryo because of some inimical environmental factor." As NOSSAL AND MÄKELÄ point out, this extension of the clonal selection theory is in some respects similar to one proposed earlier by LEDUC, COONS AND CONNOLLY (64). Moreover, all the facts revealed and all the assumptions made, can also be explained on an "instructive" basis. It is as yet not possible to clone antibody forming cells *in vitro* without loss of their function. Consequently no