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Editor

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PREFACE

A Concept of Modern Immunology

J. B. G. KWAPINSKI (*Editor*)

Because of the rapid development of Immunology, simultaneously in width and depth, it has become increasingly difficult to define and delineate this branch of science. Immunology can be broadly described as a biological science, concerned with the bodies and phenomena which occur in a subject upon contact with molecules or other structural units possessing a specific configuration and conformation.

Immunological research and study deal with both the theory and practice of immunology. Theory and fundamental research are devoted to the elucidation and description of immunological phenomena and mechanisms, the origin and nature of antigens and antibodies and of their interactions *in vitro* and *in vivo*. The practice of immunology deals with serological and immunochemical methodology and its diagnostic and prognostic applications, as well as with immunization.

Immunology can be divided, for convenience, into three large branches: immunobiology (immunity), immunochemistry, and serology. Immunobiology deals with the origin, inducement, and mechanisms of immune response (immunity). Immunochemistry is concerned with the chemical synthesis, constitution, and molecular interactions of antigens, antibodies, and the host as a whole. The field of serology deals primarily with antibodies and their reactions with antigens outside the host. Such a division of immunology, which is only justified by convenience, would have to confront and accommodate such important areas of the immunological endeavor as immunogenetics, molecular and quantum immunology, and molecular and quantum immunochemistry. Before long the latter discipline, a very modern science, should set trends for the theoretical and practical aspects of modern immunology, with quantum immunochemistry providing long-awaited information as to the nature of immunological response, synthesis of immunoglobulins, and the antigen-antibody reaction.

In this view (KWAPINSKI, unpublished), immunological specificity depends on a quantum of energy existing in a particular space and time in a conformational area of a nascent molecule site. This definable (specific) quantum of energy is transmitted by electrical contact to the conformationally complementary sites of certain host-molecules, charging them in accordance with the conformation of the inducer-molecule, so that in time sufficient numbers of such immunologically conformant molecules are present to be detected by gross serological tests, in which the charge is lost. The original quantum of energy is usually exhausted after a time, although it may persist in some cells to be "reactivated" by a secondary stimulus of an identical conformational unit, thus giving impetus to a second, chain-like energy reaction, which results in the accumulation of immunologically active and detectable bodies (antibodies).

Since immunology is closely linked to, and heavily based on biochemistry, physical chemistry, biophysics, microbiology, pathology, molecular biology, and genetics, its rapid development in recent years has been reflected by an ever-increasing number of publications dealing entirely or in part with various aspects of this group of disciplines, as well as with immunology proper. The significance of these publications usually remains concealed until they are evaluated and interpreted in terms of our current knowledge of immunochemistry and immunobiology.

The objective of this new series of scientific publications entitled RESEARCH IN IMMUNOCHEMISTRY AND IMMUNOBIOLOGY is to present significant ideas and data on various subjects of immunochemistry and immunobiology, obtained from the research work and experience of leading scientists, and supplemented by a comprehensive yet concise and critical discussion of the pertinent literature. Although both specialized and broad knowledge of different aspects of immunochemistry and immunobiology will be balanced against each other in these books, it is our sincere desire to lead this scientific endeavor into the very presence and future of this biological science—that is, into Molecular and Quantum Immunology.

The Origin, Development, and Significance of Immunoglobulins

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The main purpose of this paper is to analyze primary factors which may affect the process of antibody formation.

I. The Antigenic Stimulus and the Origin of Immunoglobulins

A. The Immune System

The development and increase of antibody is due to active synthesis and is not caused by spilling of stored antibodies. Not all cells capable of synthesizing proteins are involved in antibody production. This knowledge is based in part on the empirical observation that antibody production may be suppressed when certain tissues are removed or when treatment with certain drugs has been administered. Potentially antibody-producing cells are widely distributed in the body of mammals and of man. The site at which antibody formation actually occurs depends on several factors, such as modus and site of immunization, amount of antigen, use of immunological adjuvants. The total of cells responsible for antibody production may be designated as "immune system" [151]. This system consists of the lymphoepithelial thymus, bursa of Fabricius in birds, lymph nodes, spleen, bone marrow, tonsils, appendix, Peyer's patches, and numerous lymphoid aggregates. The tissues harbor a variety of cells, i.e., macrophages, plasma cells, and lymphocytes which are involved in numerous steps of the immunoglobulin synthesis. Macrophages are designated as antigen-trapping cells, whereas lymphocytes and plasma cells are the producers of the immunoglobulins proper. The macrophages represent by far the largest cell type. This evidently corresponds with its function of taking up cell debris and components or whole cells up to the size of erythrocytes and polymorphonuclear leukocytes. For this task of processing antigens and of transferring information to the immunologically competent cells, macrophages possess numerous hydrolases. Macrophages arise from stem cells of the reticular tissues or from blood monocytes which have developed from stem cells of the bone marrow. It is not yet determined whether macrophages can be transformed to plasma cells. Mature plasma cells are assumed to be end cells with a maximal life span of 2–4 days. The lymphocytes are a heterogeneous group of cells differing considerably in size. The most important type is the small lymphocyte. It is characterized by scanty cytoplasma and a round nucleus with densely packed chromatin. The so-called "small lymphocyte" probably has stored much of genetic information. Small lymphocytes are distinguished by their sensitivity to a primary or a secondary antigenic stimulus.

They may be transformed into "immune cells" which synthesize antibodies without secreting them, thus being able to function as effector cells in delayed hypersensitivity responses. In mice, small lymphocytes have been identified as precursors of erythropoiesis [63]. Immune cells are present in the lymphoid tissues in variable quantity and frequency. The immune system is divided into primary and secondary lymphoid organs. The primary system is represented by the thymus in all vertebrates and the bursa of Fabricius in birds, whereas the secondary lymphoid organs comprise all other lymphoid tissues except the bone marrow which has a special position as the site of stem cells. This classification is based on the fact that in the thymus and bursa of Fabricius, lymphocytes appear earlier than in the secondary lymphoid tissues.

1. Primary Lymphoid Organs

Ontogenetically, the thymus is the earliest lymphoid organ, which develops from the third branchial pouch. In the thymus of mice, earliest lymphocytes appear during the second week of pregnancy. Subsequently, most of the thymic cells become large, medium, and small lymphocytes, showing intense mitotic activity. This evolution is observed in both the germ-free animal and the fetus suggesting that the intense lymphopoiesis is antigen-independent. Although the earliest lymphocytes are detectable in the thymus, it is conceived that the lymphocytes develop stepwise by differentiation from stem cells which enter the thymus at the beginning of the second half of embryonic life. Stem cells are descendants of blood islands in the embryonic yolk sac and are later demonstrable in the bone marrow [249]. Stem cells are assumed to be multipotent, functioning as common ancestors for granulocytes, lymphocytes, megakaryocytes, and erythrocytes. Lymphocytes migrate subsequently from the thymus into spleen and lymph nodes, where they form colonies of lymphoid cells. The bone marrow takes part in the lymphoid colonization of secondary lymphoid organs. In contrast to lymphocytes of secondary lymphoid organs, thymus lymphocytes do not appear to be capable of producing noteworthy amounts of antibodies. Thymus lymphocytes possess a special surface antigen not present on the lymphocytes of either the spleen or the lymph nodes. The importance of the thymus for immunological phenomena was deduced from clinical observation that benign thymoma may be associated with immunological deficiency [140]. The same applies to congenital fail-

ure of the thymus. The results of experimental thymectomies in laboratory animals, mainly in mice, were investigated by many workers and gave considerable insight into thymic function [8–10, 12, 17, 62, 176, 219, 222–226, 228, 257, 314, 339]. Neonatal thymectomy does not lead to comparable effects in all species, but is rather dependent on the species used. Slight effect is discernible in dogs and rabbits. Characteristic signs of immunological deficiency can be produced in numerous strains of mice and in the golden hamster. In comparison to normal controls, the neonatally thymectomized mice are much more susceptible to bacterial and viral infections, and spleen and lymph nodes appear to be reduced in size. This is associated with the striking diminution of lymphocyte contents in blood and tissues. Homografts and heterografts of skin are accepted. Depending on the mouse strains employed, after an interval of 1–4 months, a wasting syndrome sets in, and the animals die. The immunological capacity can be restored at the best by early substitution with thymic cells such as by grafting of syngenic thymic tissue. Since intraperitoneal implantation of thymic tissue in Millipore chambers is likewise effective, the thymus may produce one or more hormones responsible for the maturation of immune cells. No restoration of the immunological capacity was found after administration of syngenic bone marrow cells, whereas syngenic spleen cells exhibited restorative effectiveness.

Bone marrow cells administered into completely thymectomized mice, which were irradiated some days later preserved the life but were not able to reconstitute the immunological capacity [222]. Likewise, in human patients with Di George's syndrome, characterized by the absence of the thymus, the implantation of thymus tissue effected immunological reconstitution. This effect, however, was also induced by blood transfusion and grafting of marrow from sibling [348].

After a surgical thymectomy in adult mice, relatively discrete symptoms are detectable. The circulating lymphocyte counts decrease at a rate of about 25%, and symptoms of a wasting disease appear after a period of about 6 months. If these animals are irradiated some weeks after thymectomy, the diminished lymphocyte counts persist. The mice recover very slowly, thereby showing increased susceptibility to infections. Granted that the thymus is not directly involved in immunological reactivity, it may be concluded from empirical observations that surgical removal of thymus at the time of birth may result in a striking impairment of the immunological capacity. The latter, however, is less reduced when extirpa-

tion is performed in an immunologically mature animal. Apparently, the adult possesses a reserve of immunologically competent cells. Only when this is exhausted does immunological deficiency occur. Neonatal thymectomy in rats impairs the cell-mediated immune reactions rather than the production of humoral antibodies [339]. With at least two separate immunological systems recognized, one characterized by delayed hypersensitivity reactions, and the other manifested by the production of circulating serum antibodies, the question arises whether both capacities are thymus-dependent. In chicken, the capacity for delayed hypersensitivity reactions and for antibody formation is under the control of different tissues, namely, the thymus and the bursa of Fabricius. The latter organ is a lymphoepithelial organ of birds which evolves from an epithelial structure of the endoderm. It is likewise postulated that the earliest lymphocytes demonstrable in the bursa of Fabricius do not originate from epithelial cells of the bursa but that they or their precursors immigrate from outside. Extirpation of the bursa of Fabricius in newly hatched chickens leads to an inability of producing antibodies against primarily or secondarily administered antigens [138]. This observation was confirmed repeatedly [254, 325]. Yet the bursectomized chickens were able to accomplish cell-mediated immune reactions. Accordingly, bursectomized chickens effect graft rejection and delayed hypersensitivity reaction, and develop experimental "allergic" diseases such as experimental "allergic" encephalomyelitis. Reversely, cell-mediated reactions are prevented when chickens are thymectomized at hatching. In mammals, mainly cell-mediated reactions are under the direct control of the thymus and are thus considered as thymus-dependent. The lymphoid tissues along the gastrointestinal tract [48] and the tonsils in man [151] have been suggested as possible bursa equivalents. Thymectomy may, however, impair serum antibody production in mammals. This is evidently related to a general function of the thymus as an antigen-recognizing cell pool. The necessity of a certain cooperation between thymus-dependent and thymus-independent cells is at least true for some antigens such as foreign erythrocytes.

2. Secondary Lymphoid Organs

The thymus and the bursa of Fabricius are characterized by the antigen-independent lymphopoiesis. In the secondary lymphoid organs, lymphopoiesis is normally antigen-dependent. The secondary lymphoid

organs contain a mixture of thymus-dependent and thymus-independent lymphocytes. The latter cells are assumed to have developed under the control of the bursa of Fabricius or a bursa-analogous tissue. In the spleen, thymus-dependent lymphocytes are localized in the periarteriolar lymphocyte sheath, and in the lymph nodes they reside in the paracortical area. In part they belong as small lymphocytes to the recirculating lymphocyte pool. Thymus-independent lymphocytes are localized in the lymphoid follicles and in the germinal centers. These tissues represent essential factories for the synthesis of immunoglobulins and the production of immune cells.

B. The Antigenic Stimulus

The necessary prerequisite for production of immunoglobulins in man and animals is the antigenic stimulus. Large numbers of substances functioning as antigens are present in the live and lifeless nature. Many synthetic antigens have been produced [292, 293]. The immune response is not always or exclusively characterized by the appearance of circulating serum antibodies. The antigenic stimulation may also lead to the production of immune cells which play an important role either in certain defense mechanisms of the body, such as a rejection of tissue transplants, or may cause clinical manifestations, e.g., on the basis of existing delayed contact-type skin sensitivity. The immune cells involved in such reactions possess antibody-like receptors on their surfaces directed against the corresponding antigens.

1. Structure of Antigens

Originally the term "antigen" was used for material which after injection into an animal was capable of eliciting antibody formation. To this effect, antigens were presumed to possess three essential properties: large molecular weight, protein nature, and foreignness for the body to be immunized. These essential features are accepted nowadays only in part for characterizing the conditions at which a substance may be able to induce antibody production. It is well known that not only proteins but also carbohydrates, nucleic acids or their complexes elicit antibody formation. It should be kept in mind, however, that the meaning of the term "anti-

genicity" has changed after it has more and more been used for the description of antigenic specificity. Thus antigenicity circumscribes two different activities of antigens: (1) the capacity to induce the formation of antibodies with corresponding specificity; (2) the ability to react specifically with the antibodies formed.

According to SELA [292] the first of the two activities is called "immunogenicity," whereas the second may be designated as "antigenic specificity."

Evidently the factors responsible for the immunogenicity of the material have little to do with those contributing to antigenic specificity. For example, the immunogenicity of O antigens from gram-negative bacteria is effected by a protein, while their immunological specificity is bound to a polysaccharide [235, 236]. Immunization with *Bacillus anthracis* elicits antibodies which are specifically bound by poly-γ-D-glutamic acid, a cell wall constituent of these organisms [174, 273]. Likewise, on the molecular level, immunogenicity and antigenic specificity must not necessarily depend on identical areas of the molecule. This had already been demonstrated by the pioneering studies of LANDSTEINER [1965] on the specificity of serological reactions. Simple chemical compounds with low molecular weight were attached artificially to proteins, e.g., by a diazo linkage. The injection of such conjugated proteins into animals resulted in the formation of antibodies directed against the carrier protein and led further to the formation of immunoglobulins specifically directed against the attached small molecules. Consequently, the antibodies are not produced against the whole complex of conjugated protein but against defined structural areas of the antigen. Substances which are able to change the antigenic specificity of macromolecules and possess the capacity of reacting specifically with the immunoglobulins formed against the conjugated proteins, are called haptens. In natural antigens, such as proteins and carbohydrates, the specific antigenicity also depends on certain structural features of relatively small areas. These are called "antigenic determinants." Functionally the same property is expressed by the words "hapten" and "antigenic determinant," although the two designations cannot be considered as identical. In some artificial antigens the determinant area is represented by the hapten and in addition by a small part of the protein carrier [80]. This holds true only when the attached hapten is smaller than a tetrapeptide [294]. This means that in such cases the hapten is smaller in size than the determinant. It serves then only as its immunodominant part.

Natural antigens usually possess several identical determinants functioning as the specific antibody-combining sites. The term "valency" describes how many antibody molecules could be bound by an antigenic molecule. On steric reasons only a limited number of antibody molecules can be bound. It follows that the antigenic determinants of an antigen may be more numerous than its experimentally determined valency would indicate. This accounts for the insufficient knowledge of the true number of antigenic determinants present in antigens, as is evident from the following example:

The consensus is that antigens should be at least bivalent to form with corresponding antibodies a specific precipitate or agglutinate. The immunogenicity of insulin is well known. But antibodies produced against it would not precipitate. This was explained on the basis that one antibody-combining site exists per molecule only. This univalent character of insulin would not allow the construction of an antigen-antibody-lattice [32–35, 170 189]. However, the strongly positive results obtained by means of several other serological procedures, such as the hemagglutination and complement fixation tests, did not completely fit in this explanation. Dating from 1960 it was claimed that antisera did form precipitates with insulin [98, 263]. Those findings were not generally accepted because LAPRESLE [197] had warned that even crystalline insulin preparations may still contain antigenic impurities. The precipitation could have been effected by a reaction between the impurity and its corresponding antibody [197]. On the other hand, antibodies produced in guinea pigs against cristalline preparation of pig insulin reacted with the homologous material and in addition with a native insulin obtained from another source as well as with a bovine insulin (fig. 1). The same was found with antisera directed against bovine insulins. But antisera against native or photooxidized [1] preparations of either bovine or pig insulins did not precipitate oxidized [2] insulin or isolated A- and B-chains [119, 120, 285]. The main evidence for the exis-

[1] Radiation of insulin was performed according to the method described by WEIL *et al.* [343, 344] concerning the photochemical disruption of amino acids by molecular oxygen in the presence of methylene blue as catalyst. This procedure leads to the disruption of the imidazole rings of histidine without influencing steric conformation.

[2] The oxidative separation of insulin into the sulfonates of the A- and B-chains was done as described by VOELKER *et al.* [336]. The completeness of the separation was proved by means of paper electrophoresis using 0.05 M veronal buffer (pH 7.4).

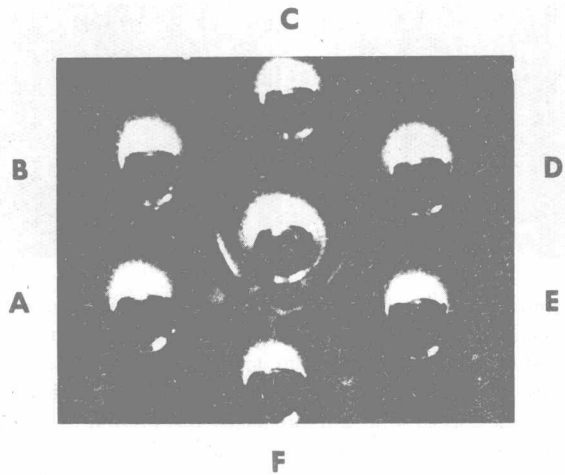

Fig. 1. Immunodiffusion reaction between a guinea-pig antiserum produced against pig insulin (the central well) and native bovine insulin (A), bovine serum albumin (B), mixture of isolated A and B chains of bovine insulin (C), oxidized pig insulin (D), photooxidized pig insulin (E) and native pig insulin (F) [98].

tence of insulin-antibody-precipitates is derived from the observation that photooxidized insulin has a faster electrophoretical mobility than native insulin. This is explained by the fact that the photooxidized preparation in comparison to native insulin has a precipitation range which is shifted by about 1.5 pH units toward the acid side. Accordingly, the precipitates formed with photooxidized insulins were constantly found to be localized more anodically than those formed with native preparations of either pig or bovine insulin (fig. 2). On the basis of these experiments one can hardly maintain that insulin represents a univalent antigenic structure.

The true number of antigenic determinants may also be masked by the fact that an antigen such as albumin may have several different antigenic determinants, thus eliciting antibodies with different specificities. All sorts of cells including those of fungi, bacteria, and viruses which are often called antigens, actually represent mixtures of numerous polydeterminant antigens.

The specificity of antigenic determinants of globular proteins depends on the amino acid sequence and is mainly conformation-dependent. The early studies of LANDSTEINER [195] elucidated the important role of

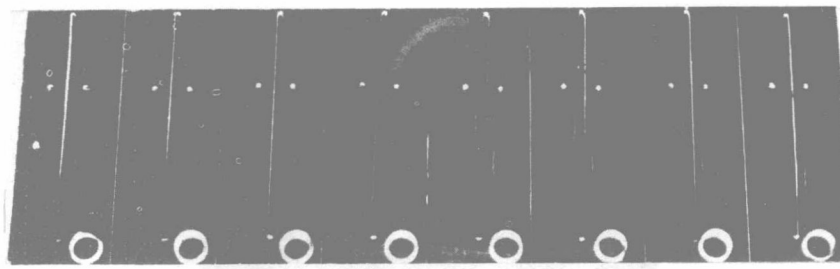

Fig. 2. Immunoelectrophoresis test between native pig insulin (placed in the left well) or photooxidized pig insulin (right well, before electrophoresis) and an antiserum. The slides show: *1* antiserum to native pig insulin; *2* antiserum absorbed with native pig insulin No. 1; *3* antiserum absorbed with native pig insulin No. 2; *4* antiserum absorbed with photooxidized pig insulin; *5* antiserum absorbed with oxidized pig insulin; *6* antiserum absorbed with A chains of bovine insulin; *7* antiserum absorbed with B chains of bovine insulin and *8* antiserum absorbed with a mixture of A and B chains of bovine insulin [98].

optical configuration of the haptens employed and proved that the antibodies formed are stereospecific. Antibodies against poly-L-alanyl determinants do not react with poly-D-alanyl determinants [13, 284]. Assuming that the antigenic determinants in globular proteins depend on the integrity of a well-defined 3-dimensional structure, it may be suggested that the characterizing steric conformation must be saved until the determinant has been recognized by those cells which serve as information receptors. The conclusion may be drawn that up to the recognition of determinants by competent cells enzymatic splitting of antigenic macromolecules may take place but only in so far as their steric conformation is preserved.

The maximum sizes of antigenic determinants functioning as combining sites, are equivalent to either those of hexasaccharide or those of a tetra- to hexapeptide [143, 155, 186, 280, 294, 310]. The antigen-combining sites of both IgG and IgM antibodies are equivalent to a tetrapeptide [155].

Knowledge of the structural conditions under which a substance may be immunogenic is even more limited. One of the classical postulates of immunogenicity' of a substance is a sufficiently high molecular weight [160, 188]. The minimal requirements were, however, steadily reduced. Nobody would doubt that particulate antigens such as bacteria and viruses are much more immunogenic than monomolecular dissolved proteins.

Among the latter group, proteins with molecular weights of 40,000 in general are good immunogens. When substances with lower molecular weight, such as ribonuclease (molecular weight of 14,000), insulin (molecular weight of 6,000) or glucagon (molecular weight of 3,800) are administered together with immunological adjuvants they will regularly elicit antibody production. Synthetic polypeptide antigens with molecular weights of 4,000 [298] and 5,000 [208] have the same effect. Synthetic antigens with considerably smaller molecular weights were produced, such as α-dinitrophenyl hepta-L-lysine (molecular weight of 1,080) [287, 289]. Smaller molecules have been identified as immunogens [2, 36, 37, 67]. Since it is not necessary that the linkage between the haptenic group and the carrier be covalent [280], the possibility cannot be ruled out that the immunogenicity of substances with very small molecular weights is due, at least in part, to their binding to the host's proteins or cells. The immunogenicity may be enhanced by attachment to an insoluble carrier [166, 253] and by aggregation of molecules to larger clumps as was shown by DRESSER [72] on the immunogenicity of bovine IgG preparations. Monomer insulin molecules also assemble to units of higher molecular weight. This process is most pronounced at the pH range 7–7.5 thus leading to molecular weights of about 36,000 [121, 286]. Thus, by aggregation of monomer insulin, molecular weights are attained which would correspond to those of powerful immunogens, such as ovalbumin (molecular weight of 40,000). Numerous further observations indicate that immunogenicity does not depend alone on the molecular weight. Serum albumin and hemoglobin possess a similar molecular weight but only albumin is usually a powerful immunogen. Gelatin, which has a higher molecular weight than ovalbumin, is a very poor immunogen, but it became highly immunogenic when 2% peptide chains of tyrosine were conjugated on to it. The antibodies produced against this preparation in rabbits and guinea pigs cross-reacted well with the native gelatin [295, 297]. When, however, 10% tyrosine groups were attached to gelatin, the antibodies formed against this good immunogen reacted exclusively with the tyrosyl peptides, but no longer to native gelatin. The conversion from a very weak to a very good immunogen was also effected when cyclohexylalanine peptides were attached to gelatin [296]. Thus, aromatic amino acids are not necessary for the enhancement of immunogenicity. The areas of a molecule to render it immunogenic must obviously be present on the outside of a macromolecule in order to be accessible [292–294]. Tyrosine groups seem to be of special importance.