Meeting report

ANTIGENS AND IMMUNOGENICITY

Joint Biochemical Meeting of the Société Belge de Biochimie – Belgische Vereniging voor Biochemie and the Gesellschaft für Biologische Chemie, Liège, January 14–16, 1971

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1. Introduction

The meeting was organized by Claude Liébecq (Liège) in co-operation with the universities of Aachen and Liège. It was supported by the University of Liège, Behringwerke AG, the Division Pharmaceutique de l'U.C.B. and Janssen Pharmaceutica n.v. Papers read in the *Peptides and Proteins* Section have already been reported on [1]. In addition, abstracts of papers presented at the meeting were published elsewhere [2].

In view of the growing importance of immunological problems for biological sciences another major part of the meeting was devoted to some of the more biochemical aspects of immunology.

2. Induction of an immune response

In the opening lecture H. Fischer (Freiburg) highlighted current concepts of the cellular and biochemical mechanisms involved in the initiation of an immune response. He started with a short survey of selective and inductive theories of antibody formation, the former being first expressed by Paul Ehrlich who assumed that antigen (toxin) is specifically bound by cellular receptors (*Haftstellen*). This binding causes the cell to produce an increasing amount of the same receptors which are finally released into the medium. These ideas already indicated what we would call in modern terms, "the problem of antigen recognition" by membrane bound cellular receptors, the triggering of cells to proliferate upon interaction with antigen, and finally the production of antibodies.

It is now generally accepted that small lymphocytes represent the immunocompetent cells. Their origin and differentiation from stem cells into what appear to be functionally distinct bone marrow-derived lymphocytes (B-cells) and thymus-derived lymphocytes (T-cells) has been reviewed [3]. The former are the precursors of the antibody forming cells while the latter are the mediators of cellular immunity and in addition can cooperate in an as yet not clearly defined way with B-cells in initiating the events finally leading to antibody production.

Another cell type which has long since been thought to play a more or less important role in immune responses is the macrophage. On the one hand macrophages are known to degrade macromolecular and cellular antigens and it has been suggested that their function is merely to remove excess antigen. On the other hand, macrophages, in particular the so-called dendritic macrophages, are known to bind antigen on their surface for a considerable time [4, 5]. This macrophage-bound antigen can possibly then be recognized in an efficient way by lymphocytes. In an excellent film it was demonstrated that lymphocytes moving across the omentum can make intensive membrane to membrane contact with such dendritic macrophages.

Furthermore by time lapse cinematographic analysis one can count the number of macrophage-lymphocyte contacts under various conditions of immunication, and demonstrate the occurrence of two types of lymphocytes which differ in their average mobility.

The outcome of the interaction of antigen with the cells of the immune system is also strongly influenced by the use of adjuvants. It has been suggested that some of these materials, which apparently help to trigger lymphocytes, act by releasing lysolecithin and lysocephalin from the membrane of macrophages. This assumption is supported by the finding that lysolecithin and some synthetic analogues when injected intraperitoneally into mice do indeed function as powerful adjuvants.

U. Hämmerling (Giessen) described his studies on the characterization and the maturation of lymphocytes by using surface markers i.e. alloantigens which can be detected by specific antisera [6]. The topographical distribution of these antigens was investigated by employing essentially three approaches: the cytotoxic test, quantitative determination of antigens by ^{3}H-labelled antibody, and the location of the antigens in the electron microscope by ferritin-labelled antibodies. It was found that the various antigens are not distributed homogeneously over the cell surface but are confined to certain areas. Lymphocytes of different origins show characteristic quantitative and qualitative differences in their antigen pattern. Thymocytes, for instance, have the TL antigen, a high content of θ-antigen but relatively little H 2-antigen and no immunoglobulin. Thymus-derived lymphocytes, on the other hand, have no TL, little θ but a larger amount of H 2-antigens. They have in addition surface-bound immunoglobulin, presumably immunoreceptors. These approaches, especially when combined with micromanipulation techniques, appear very promising for characterizing the lymphocytes actually participating in immunological reactions and to investigate their origin and way of differentiation.

Small haptenic groups are generally capable of inducing antibody formation only when coupled to a so-called carrier which for practical purposes is usually protein. K. Rajewski (Köln) reviewed evidence showing that this requirement for a carrier may be explained by a co-operation of the precursors of the cells producing anti-hapten antibody with cells recognizing determinants of the carrier molecule. With respect to the production of anti-hapten antibody the latter have been termed "helper" cells and are most probably thymus-derived lymphocytes [7]. Thus, the carrier effect is in agreement with the finding that thymus-derived lymphocytes and bone marrow-derived lymphocytes act synergistically in inducing antibody formation to a great variety of antigens.

From these results one could expect, and there is evidence supporting this assumption, that one of the minimal structural requirements for immunogenicity is the presence of at least two different determinants on an antigen molecule, one of them being a helper determinant with respect to the other. On the other hand, certain antigens, mainly those consisting of repeating units of identical determinants, appear to be thymus independent i.e. they are capable of inducing antibody formation in the absence of the thymus derived cells.

In studies with synthetic polypeptides which were summarized by E. Rüde (Freiburg) it was consistently found that more complex copolymers composed of three or four different amino acids are better immunogens than those composed of only two amino acids or than homopolymers which are usually nonimmunogenic. These results are in agreement with the requirement of different types of determinants for inducing antibody formation by cooperation of T- and B-cells, since the presence of suitable determinants is more probable in more complex antigens.

By analogy, the relatively simple structure of most polysaccharides may, to some extent, be responsible for the fact that many polysaccharides are relatively poor immunogens. Other factors may also be involved. In studies with synthetic model antigens it was found for instance that various mono- and disaccharides when bound to the side chains of a weakly immunogenic branched polypeptide (multichain poly-D,L-alanine) were not capable of enhancing its immunogenicity. On the other hand, the attachment of certain amino acids such as tyrosine or histidine to the same polypeptide has long been known to convert it into a good immunogen. From such experiments it appears that sugar molecules can function as efficient determinant groups

when bound to a carrier but usually have low immunogenic potency.

Among the most potent immunogens are those composed of a large number of identical protein subunits such as bacteriophages or the flagellae of bacteria [8]. Another example is the tobacco mosaic virus (TMV) about which F. Loor (Bruxelles) reported. In contrast to findings with other antigens of this type it seems to be extremely difficult if not impossible to induce in mice a state of tolerance towards TMV. Even injection into new-born mice of high doses of the monomeric TMV protein or of its tryptic digest, which in the flagellin system is the best tolerogenic treatment, was not regularly found to be effective in suppressing the antibody response to a challenge injection of the antigen. Explanations of this property of TMV were discussed, among them the possibility that tolerance would depend primarily on the depletion of "helper" cells. One might then expect that TMV can directly stimulate the precursors of the antibody producing cells in the absence of a helper mechanism.

Antibody affinity is known to rise during the course of an immune response. This has been clearly demonstrated for the anti-2,4-dinitrophenyl-system and has been suggested to be due to an antigenic selection mechanism [9]. J. Urbain and coworkers (Bruxelles) studied similar affinity changes in the anti-TMV and anti-bovine serumalbumin systems by using an ultracentrifugal method which does not necessitate specific isolation and possibly selection of the antibodies. In contrast to previous results an initial rise of binding affinity in these systems was followed by a decrease of affinity. But in spite of this decrease the antibodies formed upon a secondary injection of the antigen showed high affinity, and this secondary rise in affinity was again followed by a decrease. In general the affinity changes paralleled the development of antibody concentration. Possible mechanisms which could explain such results were discussed.

3. Structure and function of antibodies

The specificity of antibodies is determined by the amino acid sequence of the variable region of their heavy (H) and light (L) polypeptide chains. A relatively large number of genes (V-genes) coding for this part of the peptide chains must therefore exist. It is not known, however, if all of these V-genes are directly inherited (germ line theory) or if they originate from a relatively small number of genes by a somatic hypermutation or recombination process which occurs during ontogeny or during differentiations of stem cells to immunocompetent cells. The comparative sequence analysis of human L-chains (Bence Jones proteins) about which N. Hilschmann (Göttingen) reported is one of the main experimental approaches which can help to clarify this problem.

According to their homology in sequence human L-chains of both the κ- and λ-type can be arranged into 3 or 4 different subgroups, respectively. Proteins belonging to one subgroup differ from one another by fewer amino acid exchanges than do proteins of different subgroups. Within a subgroup most exchanges can be explained by single point mutations whereas between different subgroups the number of amino acid differences requiring two base exchanges is higher. The validity of this arrangement could also be shown by the preparation of subgroup-specific antisera which recognize antigenic determinants common to the proteins of one subgroup but not present on others. It was suggested that these sequence data can best be explained by assuming a phylogenetic origin of antibody variability, i.e. that all V-genes are already present in the germ line [10]. An evolutionary tree for the L-chains, similar to that for the various hemoglobin chains, can be constructed with the subgroups representing the main branches and the individual proteins the terminal ramifications of each branch.

Specifically purified antibodies, even when directed against a single determinant group are usually heterogeneous with respect to the structure of the variable regions of their H- and L-chains. Sequence analysis of these regions of immunoglobulins have therefore been largely confined to monoclonal pathological immunoglobulins as has been described above. However, during the last few years methods have been developed for obtaining antibodies of restricted heterogeneity by experimental immunization. One of these consists in hyperimmunizing rabbits with streptococci or with pneumococci. A few of the animals have been found to produce anti-carbo-

hydrate antibodies of high molecular uniformity [11, 12]. M. Wikler (Bruxelles) reported similar experiments but could show that a large percentage of the animals do produce antistreptococcal carbohydrate antibodies exhibiting electrophoretic patterns of no more than 2 or 3 bands. This restricted heterogenicity is also reflected in the sequence of the 3 *N*-terminal amino acid residues of the L-chains which are predominantly Ala–Asp–Val– or Asp–Val–Val– for all of these antibodies.

Some indications of a limited heterogeneity of antibodies raised by the immunization of rabbits with *Proteus vulgaris* OX 19 were discussed by G.I. Pardoe (Birmingham). When complexes of these antibodies with the bacteria were used to immunize rabbits of different allotype specific anti-allotype antibodies were obtained, whereas in rabbits of the same allotype anti-idiotypic antibodies were produced. It was suggested that a high proportion of the anti-Proteus antibodies obtained from a single animal have the same idiotypic specificity.

In spite of the heterogeneity of anti-hapten antibodies obtained by conventional immunization with a hapten-protein conjugate it has been shown in some cases that the main stuctural features of the combining site are common to most of the antibody molecules capable of specifically binding the particular haptenic group. In studies with anti-NIP-antibodies reported by M. Joniau (Leuven) the haptenic group itself could be used as a spectrophotometric probe for the combining site of anti-NIP-antibodies. It was found that the pK value of the phenolic hydroxyl group of NIP-haptens is lowered by several orders of magnitude when bound to antibodies, i.e. the hapten is bound to the active site preferentially as the phenolate anion. In agreement with these results it was found previously by chemical methods that in a very high proportion of anti-NIP antibodies the positive charge of an arginine residue is essential for the binding of NIP-haptens.

The study of the kinetics and thermodynamics of reactions between antibody and multivalent antibodies is complicated by the great number of insoluble aggregates and soluble complexes which are formed. A rate zonal centrifugation method for the separation and analysis of the soluble complexes has been developed by J. Steensgaard (Århus). Different applications of the method were discussed.

4. Structure and serological specificity of antigens

4.1. *Proteins*

R. Timpl and J. Rautenberg (München) discussed the immunological properties of collagen which in comparison to other proteins are unusual, because most of the antigenic determinants are conformation-independent. The specificity and structure of these determinants can thus be studied with relatively small peptide fragments obtained by chemical (BrCN) or enzymatic cleavage of the isolated polypeptide chains. Only a minor portion of the antibodies raised in rabbits against collagens of several other species is directed towards the triple helical middle region of collagen. The major determinants are located on the terminal region of the polypeptide chains and comprise non-helical sequences. In human and calf collagen this is mainly the *C*-terminal and to some extent also the *N*-terminal part of the $\alpha 1$ chain. In the case of rat collagen early antibodies are directed against both the *C*-terminal parts of the $\alpha 1$ and $\alpha 2$ chains and, after repeated immunication, the *N*-terminal part of the $\alpha 2$ chain becomes immunodominant The amino acid sequence of some of the serologically active peptides which are composed of 15–20 amino acids has been determined and it has been suggested that aromatic amino acids may play an important role in specificity.

The structure of the main antigenic determinant of a very interesting basic protein antigen isolated from human brain was described by R. Carnegie (Melbourne). This protein, upon injection with complete Freund's adjuvant into guinea pigs, is known to induce allergic encephalomyelitis, a cell-mediated autoimmune disease. A peptide fragment composed of 15 amino acids and a corresponding synthetic peptide, Ser–Arg–Phe–Ser–Trp–Gly–Ala–Glu–Gly–Gln–Arg, were both found to be capable of inducing a state of delayed hypersensitivity towards the original protein without detectable antibody formation. It was proposed that this peptide is part of a receptor for 5-hydroxytryptamine in a neuronal membrane.

The nature of the bovine J blood group antigen which occurs both in the plasma and on the surface of the red cells was discussed by O.W. Thiele (Göttingen). Serological activity in plasma was found to be associated with a lipoprotein and probably also

with a mucoprotein. The lipoprotein is passively absorbed onto the red cells after birth and appears to be fully integrated into the cell membrane, whereas the mucoprotein is not bound to the cells. This is suggested by the observation that in so-called J_s animals J-activity is present as mucoprotein in the serum but practically no activity can be detected in the lipoprotein fraction and on the red cells.

A similar situation may also apply to sheep red cells. P.E. Kaiser (Frankfurt) reported that the immunization of mice with sheep serum results in the appearance of cells producing γM-antibodies capable of lysing sheep red cells in the presence of complement (plaque-forming cells). By various fractionation procedures it was possible to isolate the active component as a lipoprotein having a molecular weight of 340,000 and a chemical composition characteristic of human high-density lipoproteins.

4.2. *Polysaccharides*

The serologic specificity of numerous natural antigens is determined by oligosaccharide subunits. As was reported by K. Himmelspach (Freiburg) 1-(*m*-aminophenyl)-flavazoles prepared from such oligosaccharides are useful intermediates for coupling such subunits to protein in order to obtain artificial immunogens carrying the sugars as antigenic determinants. For instance, antibodies specific for the tetrasaccharide $\begin{matrix}\text{Glc}\\ \text{Gal}\end{matrix}$–Man–Rha which represents the repeating unit of *Salmonella illinois* polysaccharide were obtained in rabbits using the flavazole technique. An alternative route to artificial immunogens consists of coupling aldonic acids to 2-(*p*-nitrophenyl)-ethyl-amine followed by reduction and conjugation with protein via diazotization.

Both techniques can be modified as to obtain radio-labelled oligosaccharides for use as haptens in binding studies. For this purpose 1-(*m*-hydroxyphenyl)-flavazoles or aldonic acid-tyramine conjugates are prepared. Both kinds of derivatives readily lend themselves to radioiodination.

Antigenic structures of various specificities have been detected on the surface of the slime mould *Dictyostelium discoideum* by G. Gerisch and H. Beug (Tübingen). This organism is known to develop in two phases: In the single-cell stage the cells grow and multiply, while after exhaustion of the food supply the cells, guided by a chemotactic mechanism, begin to aggregate and finally form a multicellular fruiting body.

Depending on the use of heated cells or of a water soluble fraction of cell homogenates for the immunization of rabbits, antisera were obtained which were directed primarily against carbohydrate or protein determinants of the cells, respectively. It was demonstrated that these antibodies can be successfully employed to study the surface components which participate in intercellular contact formation during the process of cell aggregation. Thus, the univalent Fab-fragments of anti-homogenate antibodies were found to be capable of completely inhibiting cell aggregation but did not affect the chemotactic receptors. On the other hand, Fab-fragments of antibodies produced by immunization with heated cells did not influence cell aggregation, although they were also bound to the cell surface. By using such test systems the sites responsible for contact formation can be identified and possibly isolated from membrane preparations of the aggregation competent cells.

References

[1] H. Zahn and H. Klostermeyer, FEBS Letters 14 (1971) 73.
[2] Hoppe Seyler's Z. Physiol. Chem. 352 (1971) 1.
[3] A.J.S. Davies, E. Leuchars, V. Wallis and M. Doenhoff, Proc. Roy. Soc. London B 176 (1971) 369.
[4] E. Kölsch and N.A. Mitchison, J. Exp. Med. 128 (1968) 1059.
[5] E.R. Unanue, B.A. Askonas and A.C. Allison, J. Immunol. 103 (1969) 71.
[6] U. Hämmerling, T. Aoki, H.A. Wood, L.J. Old, E.A. Boyse and E. de Harven, Nature 223 (1969) 1158.
[7] K. Rajewsky, Proc. Roy. Soc. London B 176 (1971) 385.
[8] G.L. Ada, C.R. Parish, G.J.V. Nossal and A. Abbat, Cold Spring Harbor Symp. Quant. Biol. 32 (1967) 381.
[9] G.W. Siskind and B. Benacerraf, Advan. Immunol. 10 (1969) 1.
[10] N. Hilschmann, H.U. Barnikol, M. Hess, B. Langer, H. Postingl, M. Steinmetz-Kayne, L. Suter and S. Watanabe, in: Current Problems in Immunology, eds. O. Westphal, H.E. Boch and E. Grundmann (Springer-Verlag, Heidelberg, 1969) p. 69.
[11] R.M. Krause, Federation Proc. 29 (1970) 59.
[12] E. Haber, Federation Proc. 29 (1970) 66.