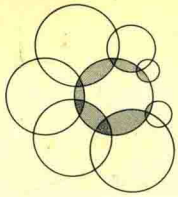
3rd International Convocation on Immunology
Buffalo, N.Y., 1972



Specific Receptors of Antibodies, Antigens and Cells

Editors:
David Pressman, Buffalo, N.Y.
Thomas B. Tomasi, Jr., Buffalo, N.Y.
Allan L. Grossberg, Buffalo, N.Y.
Noel R. Rose, Buffalo, N.Y.

S. Karger
Basel · München · Paris · London · New York · Sydney

ISBN 3–8055–1372–0

3rd International Convocation on Immunology, Buffalo, N.Y., June 12–15, 1972

Specific Receptors of Antibodies, Antigens and Cells

Editors:
D. Pressman, T. B. Tomasi, Jr., A. L. Grossberg,
N. R. Rose, Buffalo, N.Y.

With 97 figures and 79 tables

S. Karger · Basel · München · Paris · London · New York · Sydney 1973

Organizing Committee of the Third International Convocation on Immunology
D. PRESSMAN
T. B. TOMASI, Jr.
A. L. GROSSBERG
J. F. MOHN
N. R. ROSE

Previous Volumes
1st Convocation, Buffalo, 1968
International Convocation on Immunology
Editors: N. R. ROSE and F. MILGROM (Buffalo, N.Y.)
XXXVIII + 361 p., 139 fig., 39 tab., 1969
ISBN 3-8055-0888-3

2nd Convocation, Buffalo, 1970
Cellular Interactions in the Immune Response
Editors: S. COHEN, G. CUDKOWICZ and R. T. MCCLUSKEY (Buffalo, N.Y.)
VIII + 310 p., 106 fig., 93 tab., 1971.
ISBN 3-8055-1202-3

S. Karger · Basel · München · Paris · London · New York · Sydney
Arnold-Böcklin-Strasse 25, CH-4011 Basel (Switzerland)

ISBN 3-8055-1372-0

Acknowledgements

The Committee for the Convocation gratefully acknowledges contributions from the following:

Basel Institute for Immunology; Ciba Pharmaceutical Co.; Dade Division of American Hospital Supply Co.; Hyland Division of Travenol Laboratories, Inc.; Hooker Chemical Corporation; Kallestad Laboratories, Inc.; Merck, Sharp & Dohme Corporation; Ortho Research Foundation; Schering Corporation; S. Karger AG.

Table of Contents

Binding Sites of Antibodies

Antibodies of Limited Heterogeneity and Myeloma Proteins with Antibody Activity

Cellular Sites of Antigen Recognition I

Cellular Sites of Antigen Recognition II

Antibody Production and Regulation

Introduction

This volume represents the proceedings of the third of a continuing series of biennial convocations sponsored by The Center for Immunology of the State University of New York at Buffalo. The original purpose, that of bringing together scientists from around the world to present the latest findings and to discuss future trends in various areas of immunologic research, has been maintained.

The first convocation was held in Buffalo, N.Y., in June 1968, to celebrate the founding of the Center. Major areas of modern immunological research were reviewed, with emphasis upon those fields of special interest to the founder and first director of The Center for Immunology, ERNEST WITEBSKY. The topics included tissue and cellular antigens, autoimmunity and cancer immunology. The second convocation, in June 1970, explored in depth a particular facet of immunological investigation, cellular interactions in the immune response.

For the third convocation, the organizing committee again sought a topic ripe for penetrating discussion. Not surprisingly, they selected the properties of the specific sites on immunoglobulin molecules responsible for their reactions with antigen and of the specific receptors on cells whose interactions with antigen trigger the immune response. These topics are at the core of immunology, for they relate to the most significant feature of the immune response, its specificity. Much has been learned in the past few years of the properties of antibodies that allow their selective combination with particular antigens, including their physical and chemical basis, genetic control and biosynthetic pathways.

Equally fascinating are the studies of the abnormalities of the immunoglobulins, the understanding of which has elucidated an important area of

pathological physiology and taught us so much about the biochemistry of normal immunoglobulin molecules. Not only do the immunoglobulins bear powers of specific recognition, but immunologically active cells, the lymphocytes, possess an analogous ability. The comparison of immunoglobulin recognition and cell recognition was another major facet of discussion. Finally, attention turned to the control mechanisms governing both humoral and cellular immune responses.

The guest of honor at the Convocation was PIERRE GRABAR. It is rare in these days of mass-produced research for one area to bear the imprint of a single individual. In the judgement of the organizing committee, GRABAR deserves the credit for first viewing the antibody molecule as a subject for detailed physicochemical, biochemical and immunochemical analysis. The discoveries of GRABAR and his students, and especially their introduction of immunoelectrophoresis two decades ago, gave genesis to the observations under consideration.

Many of the most prominent contributors to studies of the immunoglobulin molecules benefited directly from GRABAR's inspiration. More than 150 scientists worked in his department at the Pasteur Institute or at Villejuif. Approximately one fifth of them were Americans – further evidence of the internationality of science.

I wish to thank Mrs. ETHEL LEE HELFFENSTEIN for assistance in preparing these proceedings and the publishers, S. KARGER, for their cooperation.

NOEL R. ROSE, M.D., Ph.D.,
Director, The Center for Immunology

Ernest Witebsky Memorial Lecture

Specific Receptors of Antibodies, Antigens and Cells. 3rd Int. Convoc. Immunol., Buffalo, N.Y., 1972, pp. 4–30 (Karger, Basel 1973)

General Features of Antibody Molecules[1]

E. A. KABAT

Departments of Microbiology, Human Genetics and Development and Neurology, College of Physicians and Surgeons, Columbia University, and Neurological Institute, Presbyterian Hospital, New York, N.Y.

I feel deeply honored to have been chosen to deliver the Ernest Witebsky Memorial Lecture. My first recollection of ERNEST dates from about 1934, following his arrival from Germany and during his visit with MICHAEL HEIDELBERGER while I was a laboratory assistant and graduate student. My first real contact with him dates from a long train ride in 1939 or 1940, when we sat together talking for a good many hours and during which he invited me to give a seminar at Buffalo. We became very good friends and our areas of interest overlapped extensively and included the nature of the Wassermann antigen, organ-specific substances, heterogenetic antigens, autoimmune diseases and blood groups and blood group substances to which he made fundamental contributions crucial to the development of modern immunology. We seldom saw eye to eye about how to attack a problem and invariably had spirited discussions, but generally ended up in agreement on essential findings. Although we would never admit it to one another, we each recognized the value of the other's approaches. This is the only time I have ever come to Buffalo without seeing ERNEST. The Center for Immunology and these Convocations will continue his work.

1 Aided by grants from the National Science Foundation (GB-25686 and GB-35243x) and a General Research Support grant from the United States Public Health Service.

The following abbreviations are used for amino acids:

Arg Arginine	Gly Glycine	Pro Proline
Asn Asparagine	Ile Isoleucine	Ser Serine
Asp Aspartic acid	Leu Leucine	Thr Threonine
Cys Cysteine	Lys Lysine	Trp Tryptophan
Gln Glutamine	Phe Phenylalanine	Val Valine

The title of my talk 'General features of antibody molecules', which was suggested by the committee, is especially appropriate. For several decades since the development of quantitative immunochemistry by MICHAEL HEIDELBERGER and his school and the recognition of the remarkable differences in immunological specificity by KARL LANDSTEINER, the predominant concern was with antibody heterogeneity, the seemingly limitless capacity of the individual to produce molecules with different kinds of specific receptor sites, which also differed in many of their physical, chemical and immunological properties including molecular weight, electrophoretic mobility, end groups, antigenic determinants, etc. There were indeed relatively few features apart from specificity which one could have called 'general', at least in the sense of imparting a fundamental point of view. Only during the past 12–15 years has a pattern begun to emerge that permits us to appreciate the uniqueness of antibodies as compared with other proteins, and the remarkable preservation over evolutionary time of the antibody-forming mechanism, whatever it may eventually turn out to be.

Antibody molecules and immunoglobulins may be distinguished from all other known proteins with specific receptor sites by several features:[2]

(1) The antibody-combining site is formed by two chains both of which contribute complementarity-determining residues located in the amino terminal quarter of the heavy chain and the amino terminal half of the light chain (fig. 1) called the variable region [55], [cf. 18, 41, 67, 77, 78]. The fundamental monomeric unit always consists of two identical light and two identical heavy chains [19, 29, 41, 67].

(2) Antibody and immunoglobulin molecules show segmental flexibility, i.e., the ability of the individual combining sites to move over great distances and to assume very different angles relative to one another [1, 26, 33, 47, 94, 126, 131]. This is accomplished by a flexible hinge between the Fab and Fc regions of the molecule (fig. 1) [84, 94, 109] and is seen most dramatically in the electron micrographs of VALENTINE and GREEN [126] (fig. 2) and is the region which is susceptible to the action of papain and pepsin [19, 67]. The five classes of immunoglobulins IgG, IgM, IgA, IgD, and IgE are all

2 The vastness of the literature makes it difficult to give primary references to all of the material discussed. Much background information, primary references and other points of view can be found in the three symposia, Nobel [77], Cold Spring Harbor [41] and New York Academy of Sciences on Immunoglobulins [78], as well as in books [67, 21], review articles [6, 18, 19, 26, 29, 47, 48, 50, 59, 61, 63, 69–71, 78, 79, 87, 88, 91, 102–104, 117, 124, 125], and the Proceedings of the First International Congress of Immunology [4]. Primary references to sequences may be found in [4, 41, 71, 77, 78, 129].

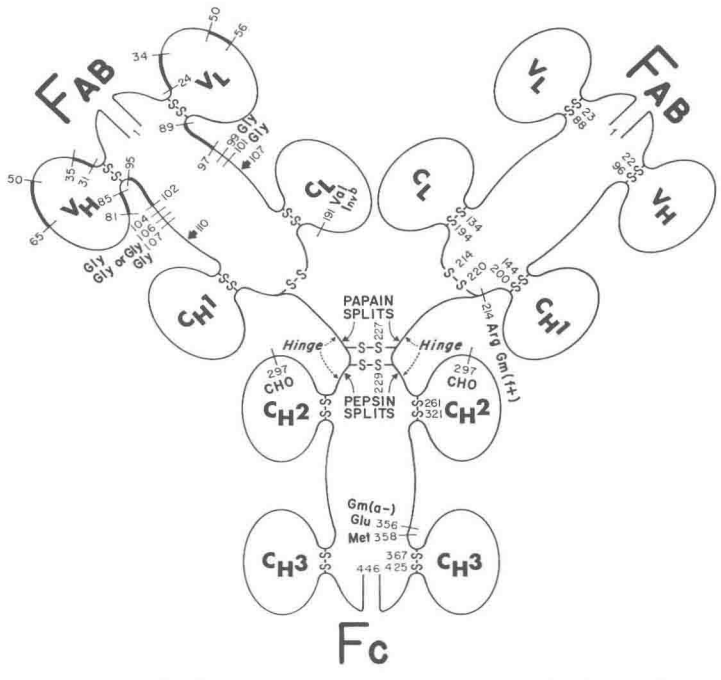


Fig. 1. Schematic view of the four-chain structure of the human IgG1κ-molecule. The numbers on the right side are the actual residue numbers in protein Eu [28]; those on the Fab fragment on the left side are aligned for maximum homology; the light chains are numbered as in WU and KABAT [129] and KABAT and WU [71]. The heavy chains of Eu have residue 52A, three residues 82 A, B, C and lack residues termed 100 A, B, C, D, E and 35 A, B. Thus residue 110, the end of the variable region, is 114 in the actual sequence. Hypervariable regions are accentuated by heavier lines. V_L and V_H are the light and heavy chain variable region, C_H1, C_H2 and C_H3 are the domains of the constant region of the heavy chain and C_L is the constant region of the light chain. The hinge region in which the two heavy chains are linked by disulfide bonds is indicated approximately. Attachment of carbohydrate is at residue 297. Arrows at residues 107 and 110 denote the transition from variable to constant regions. The sites of action of papain and pepsin and the locations of a number of genetic factors are given.

monomers or oligomers of the basic four-chain structure in figure 1, IgM and IgA having in addition a third chain, the J chain [53] which may be important in joining the monomeric subunits. IgA in secretions also is combined with another protein, the S piece [21, 125]. The basic monomeric structure exists in all vertebrate species studied and the polymeric IgM is usually a pentamer [47, 98], but it may be a hexamer in the horned toad [98] or a tetramer in certain bony fishes [17, 48] such as the paddlefish [3],

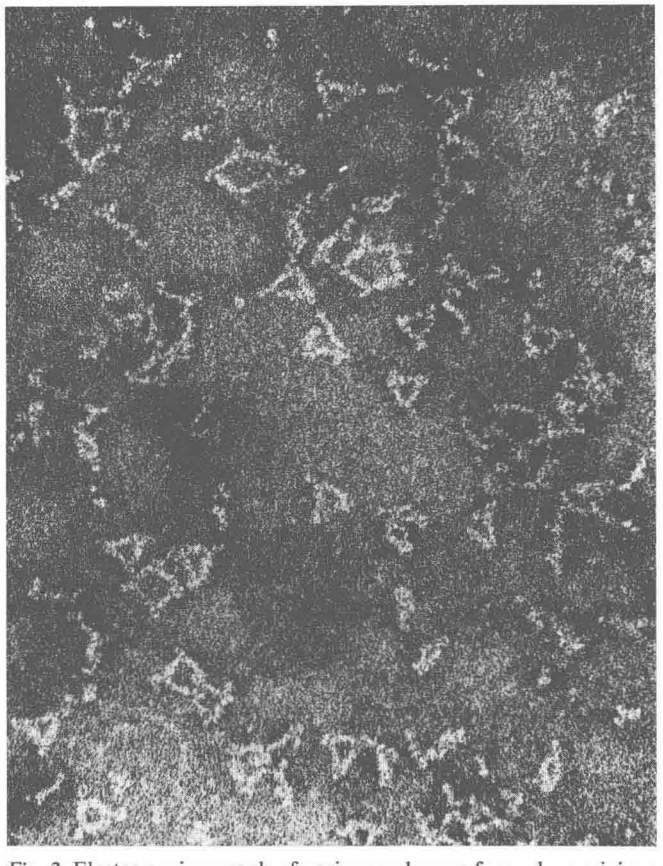


Fig. 2. Electron micrograph of various polymers formed on mixing rabbit anti-DNP IgG with bivalent DNP-hapten. Courtesy of the late Dr. R.C. Valentine [126].

margate and the grouper [8]. All show segmental flexibility in the hinge region by electron microscopy. X-ray studies [109] are also in accord with this interpretation.

(3) Antibody-combining sites vary in size and may be complementary to areas as large as that of a hexa- or heptasaccharide [46, 64, 67], the lower size limit for the complementary area of an antibody-combining site appears to be between one and two sugar residues [67]. By convention, measurements of combining site dimensions have been expressed in the most extended form but antibody-combining sites may recognize almost every shape or conformation containing similar amounts of matter. The dimensions of the hexasaccharide isomaltohexaose, molecular weight 990, in its most extended

form are $34 \times 12 \times 7$ Å and α-DNP heptalysine, molecular weight 1,080, which has been found to be antigenic by itself [112], measures $30 \times 17 \times 6.5$ Å [67]. It is not clear whether the site itself may vary in size or whether the site is always a groove of relatively uniform dimensions but with the complementarity-determining residues varying in location and in their contribution to the binding energy. A certain part of the antigenic determinant always seems to be bound most strongly: this is called the immunodominant group; it is often the terminal nonreducing sugar residue or the terminal amino acid, [46, 64, 113]. The relative binding contribution of the remaining residues decreases proportionately with the distance from the immunodominant group. In linear polysaccharides or polypeptides a nonterminal residue may also function as an immunodominant group. Receptor sites on enzymes [2] lectins and animal hemagglutinins [70] also fall within this size range but are generally homogeneous. Antigenic determinants may be sequential as in linear polysaccharides or polypeptides, or may be conformational and involve structures brought into proximity by folding as in proteins [113].

(4) The antibody response to an antigen is generally heterogeneous, since proteins, heteropolysaccharides and heteropolypeptides usually contain several different antigenic determinants. However, even to a simple homopolysaccharide such as a dextran, built up almost entirely (96% of α-1,6 linkages), the antibody response is not uniform, and antibody molecules with complementarity to different numbers of glucose residues are formed, a heterogeneous population thus being obtained. Such populations of antibody molecules may be fractionated and shown to differ in their relative reactivity with small and large oligosaccharides [67, 111]. Similar observations have been made with branched polypeptides with determinants composed of a single amino acid [110].

(5) Although only limited data are available, there does not appear to be any difference in the kinds of sites present on IgG and IgM molecules and both can give rise to antibodies with similar ranges in size, binding affinity and site complementarity [52, 93]. Association constants for antibody-hapten interaction vary over a wide range from 10^3 to 10^9 kcal/mol [73]. Association constants for anticarbohydrate antibodies tend to be lower than those toward hydrophobic residues such as the dinitrophenyl (DNP) group. The multivalence of antibody increases the effectiveness of binding [74, 88, 107]. The heavy chain generally contributes most of the binding energy [19] but light chains may also contribute significantly [62, 106].

(6) Myeloma globulins and Waldenström macroglobulins represent selected monoclonal products derived from the progeny of a single neo-

plastic immunoglobulin-secreting plasma cell. Injection of paraffin oil into BALB/c mice gives rise to myelomas resembling those of man and provides a very important experimental system [20, 102, 103]. Many of them, especially the mouse myeloma proteins, have been found to have antibody activity for DNP groups [31], dextran, levan, β-linked terminal nonreducing N-acetyl-D-glucosamine [127], β-linked terminal nonreducing N-acetyl-D-mannosamine [108], serum lipoprotein, immunoglobulin, streptolysin, various lipopolysaccharides of Gram-negative microorganisms [102], etc., the receptor sites on most myeloma globulins have not been identified. Myeloma proteins are homogeneous with respect to both their specific receptor sites and other physicochemical and antigenic properties and represent the result of selection by the neoplastic change of one of the many different immunoglobulin-producing cells. A recent study [132] of 275 human myeloma proteins tested with 20 antigens yielded none with recognizable antibody activity.

(7) Bence Jones proteins are light chains of immunoglobulins [29, 30], an excess often being synthesized and excreted in the urine. Antigenically, they fall into two major subclasses, κ and λ. From a study [105] of the tryptic polypeptide fingerprints of 101 human Bence Jones proteins, no two were found to be identical, indicating that perhaps 4,000 different light chains exist. Mouse κ-myeloma proteins also show great heterogeneity but mouse λ-proteins are extremely uniform; seven with light chains of identical sequence having been described [5, 128]. Human myeloma proteins of all five classes have been isolated [41, 77, 78]; in the mouse, IgA myeloma proteins predominate [102]. Myeloma proteins have recently been described in the dog and cat [60]. Subgroups of the variable regions of κ- and λ-chains have been described and these appear to be nonallelic; subgroups may be based on structural [58, 89, 96] and on antigenic differences [121, 122]; antigenic similarities are also seen [85].

(8) Although the antibody response is most frequently heterogeneous, by using antigens of limited complexity such as polysaccharides, dextran, levan, streptococcal C carbohydrate and the pneumococcal type-specific carbohydrates and certain polypeptides [92, 130], populations of antibody molecules of restricted specificity, some of which occasionally appear to be monoclonal, can be obtained in certain individual humans [133] or animals [50, 56, 79, 114]. Thus the anti-levan found in one person was 100% IgG2κ with over 85% of the molecules having the genetic marker Gm *n* [133]; examination of the quantitative precipitin curves with various levans showed behavior compatible with homogeneity. Individual rabbits produced anti-*p*-

azobenzoate antibodies of restricted heterogeneity; in one instance two homogeneous antibodies, differing 100-fold in binding constant, were present [56].

(9) Idiotypic or individual specificity is associated with the variable regions of immunoglobulins, myeloma globulins as well as antibodies [7, 79, 81, 82, 97, 103]. Although the individually specific or idiotypic determinants involve or are close to the site, and are influenced by the site region, they show a diversity above and beyond site specificity. Thus three individually specific antisera to human antibody to blood group A substance reacted only with the anti-A of the person from whom it was prepared and not with the anti-A of 27 other individuals [67, 83]. Antibodies of restricted specificity in humans, rabbits and mice often show a single idiotypic or individual specificity. In some instances, the idiotypic site is altered by reaction with hapten [9, 118]. Idiotypic determinants and antibody-combining sites can be reconstituted when heavy and light chains are recombined [115].

(10) The constant region of the light chains and the three constant regions of the heavy chain, each containing its own disulfide loop (fig. 1), exist as separate domains or homology regions [7, 27, 29]. The heavy chain constant regions are thought to have arisen from a process of gene duplication from the constant region of the light chain [29, 41, 54, 77, 78] and each homology region developed functions unique to itself during evolution. Each constant region has a number of genetic markers or allotypes which characterize it. Thus the κ-light chain constant region has Inv a and Inv b with leucine and valine at position 190. The Cγ1 homology region of IgG1 molecules has the Gm *f* and Gm *z* markers. Gm *n* and Gm *g* are in the Cγ2 homology region of IgG2 and IgG3 molecules, respectively, while IgG4 has Gm 4a and 4b in the Cγ2 and a marker called pFc′ in the Cγ3 domain [41, 95]. Numerous other genetic markers are also distributed within the individual domains; certain of these are dependent on the conformation of the molecule. λ-Chains appear to have undergone gene duplication with a nonallelic amino acid substitution at position 190 the Oz determinant, with lysine in Oz+ and arginine in Oz− chains; all individuals contained both chains. Allotypic markers have also been extensively studied in other species, notably the mouse and rabbit [77, 78, 124].

(11) There is general agreement that the variable and constant regions of light and heavy chains are each under the control of two genes and that they are joined together by a translocation mechanism [29, 42, 57, 91]. It may be of great significance that the variable region of a κ-chain is always joined to the constant region of the κ-chain and the variable region of a λ-chain is