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The Immune System

Edited by F. Melchers and K. Rajewsky

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Prof. Dr. Fritz Melchers
Basel Institute of Immunology
487 Grenzacherstraße
4058 Basel/Switzerland

Prof. Dr. Klaus Rajewsky
Institut für Genetik
Weyertal 1
5000 Köln/FRG

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Preface

The cells of the immune system generate a large variety of binding sites which differ in their binding specificities and can therefore react specifically with a large variety of ligands. These binding sites are part of receptor molecules, enabling the system to react to the universe of antigens.

The classical antigen receptor is the antibody molecule, and accordingly the first session of this colloquium deals with a classical subject, namely antibody structure. Dramatic recent advances in this field make it possible to interrelate primary and three-dimensional structure both to each other and to function, i.e. the binding of antigen and possible reactions occurring in the antibody molecule upon antigen binding. The latter point is of particular interest since it may be relevant not only for effector functions of antibodies such as the binding of complement, but also for the triggering of a lymphocyte through its antibody receptor for antigen.

Whereas the structural basis of antibody variability is now largely understood, its genetic basis is still unresolved. This problem is the subject of the second session, in which we ask the question of how many structural genes code for antibody molecules and how these genes are arranged in the genome. Three unlinked clusters of structural genes appear to collaborate in the coding of antibodies, and we come across many unorthodox and challenging genetic phenomena such as one polypeptide chain being coded by two or more genes, somatic translocation of genes, and allelic exclusion. Most provocative still is the question of whether antibody variability is generated somatically rather than encoded in the germ line.

How are the genes coding for antibodies expressed at the cellular level? The third session of the colloquium deals with the immunocompetent cells, namely the lymphocytes. How do these cells recognize antigen and how are they triggered by it? How are they differentiated and specialized in terms of receptor specificity and effector function? The world of lymphocytes is divided in two, the T and B cell populations, each of which can again be subdivided into functionally distinct subpopulations. B cells are the precursors of antibody-producing cells. They carry antibodies as their antigen receptors on the surface. Each cell is committed to the synthesis of a single pair of heavy and light chain variable domains, i.e. to one antigen-binding site. T cells are responsible for reactions of cellular immunity and for the regulation of lymphocyte functions in both the T and B cell compartment. The antigen receptors of these cells are at present a matter of much debate in immunology. Most interestingly, structural genes of the antibody system and also genes in the major histocompatibility complex seem to control the recognition of antigens by T cells. We are only now beginning to understand how this may be reflected in the molecular structure of the T cell receptor for antigen. The session on immunocompetent cells would be incomplete if it did not also deal with experimental tumors of lymphocytes, which are of highest relevance as model systems for the analysis of many of the most crucial problems encountered

in the immune system, and if it did not mention the macrophage, whose relation to the immune system is obvious but has also been debated for many decades.

The subject of Session IV is the major histocompatibility complex (MHC). The products of this gene complex, which may harbour hundreds or even thousands of different genes, are of crucial importance for the regulation of T lymphocyte activities. Not only do T cells react most dramatically against products of the MHC (and this is of great practical importance in the field of transplantation), but also, as mentioned above, the MHC appears to control T cell recognition of antigens and may code for parts of the T cell receptor for antigen. Due to recent technological advances, the chemical structure of histocompatibility antigens coded in the MHC is now in the process of being elucidated, and Session IV thus allows us to be present at the stage of research in immunology at which fundamental new insights into the regulation of the immune system appear to be just ahead.

The last session deals with the immune system as a system of interacting cells and molecules. Does one lymphocyte know of the existence of other lymphocytes? It has gradually become clear over the last ten years that virtually all immunological reactions rely on the interaction of lymphocytes. As an extreme view one can imagine that lymphocyte interactions are the driving force of the immune system both during its development in ontogeny and in its reactions to foreign antigens. "Lymphocyte interactions" here implies the specific recognition of one lymphocyte by another. Such interactions could well be mediated by the very same lymphocyte surface receptors which also recognize antigen. The diversity of these receptors resembles the diversity of the antigenic universe, and it is thus not surprising that in the total receptor population of a given immune system each individual receptor finds counterparts which it can specifically "recognize".

Finally, the relevance of immunology for medicine will also be discussed in Session V. It is obvious that this is a subject of enormous practical importance and that the immune system must be of great interest for doctors in almost every field of modern medicine.

Lymphocytes fascinate the immunologist because they produce a large variety of antigen-binding molecules and because they interact with each other to form a differentiated, specialized cell system of the body which deals with the invasion of foreign material and possibly fulfills other important physiological functions. Lymphocytes, however, also obey the general rules of all cells which grow, differentiate and function. They thus attract the attention of all those interested in gene expression, cell differentiation and cell interactions in higher organisms, in particular since they are amongst the best-characterized eucaryotic cells. Many different stages of differentiation of lymphocytes are defined by a wealth of genetic and molecular markers. The most important of these marker molecules, namely immunoglobulin, is well defined at the genetic, structural and functional level, but at the same time fundamental questions concerning this fascinating protein molecule are still open at each of these levels. Most importantly, the cells of the immune system can be easily obtained and maintained as single cells. This is the basis for a quantitative biology of gene expression, cell differentiation and cell interaction which makes the lymphocyte system a powerful model system in general cell biology of higher organisms.

The central role of biochemistry in immunological research appears so obvious from this brief introduction that it needs no further speci-

fication. In the frame of the immune system, biochemists have in the past found challenging problems at the level of nucleic acids, proteins and membranes. This Colloquium will, we hope, show that the same is still true at present and will be equally true in the future.

Acknowledgments. Many people have helped us in the organization of this Colloquium: the students, who took care of the projection and recorded the talks and discussions; the secretaries, who wrote the correspondence and the manuscripts of the book; Professor Zachau and the Gesellschaft für Biologische Chemie, who provided the opportunity to organize a Colloquium on The Immune System; Professor Auhagen and Professor Gibian and their coworkers, who had the largest part of the burden of organizing the Colloquium technically; Springer-Verlag, who helped to publish the proceedings of the Colloquium within 1976. Most of all, the participating speakers, chairmen and discussants.

Mosbach Colloquia are intended to present to the members of the Gesellschaft für Biologische Chemie today's knowledge of a field of modern biology. The Colloquia are, at least in part, planned as teaching endeavors on the highest level. This becomes an almost impossible task in the case of a topic such as The Immune System, which represents a network of genetics, molecular biology, biochemistry, cell biology, physiology, medicine, mathematics and much more.

It could therefore not be our intention to arrange a program which would provide a complete survey of current research in immunology. Rather, we have tried to concentrate on a few fundamental points and on what, as we think, are the most important developments and trends in the field.

We are grateful that almost everyone whom we asked to help us in this task, accepted our invitation, and then presented "his" field of immunology in such a complete and competent way as to make this Colloquium a success.

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F. Melchers

K. Rajewsky

Contributors

Adam, G.	Fachbereich Biologie, Universität Konstanz, 7750 Konstanz, FRG
Albert, E.D.	Kinderklinik der Universität München, 8000 München, FRG
Amzel, L.M.	Department of Biophysics, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205, USA
Berek, C.	Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, 6900 Heidelberg, FRG Institut für Genetik, Universität Köln, 5000 Köln, FRG
Black, S.	Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, 6900 Heidelberg, FRG Institut für Genetik, Universität Köln, 5000 Köln, FRG
Brownlee, G.G.	MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England
Chen, B.L.	Department of Biophysics, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205, USA
Cheng, C.C.	MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England
Chiu, Y.Y.	Department of Biophysics, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205, USA
Colman, P.M.	Department of Inorganic Chemistry, University of Sydney, N.S.W. 2006, Australia
Cunningham, B.A.	Rockefeller University, New York, NY 1021, USA
Deisenhofer, J.	Max-Planck-Institut für Biochemie, 8033 Martinsried, FRG Physikalisch-Chemisches Institut der Technischen Universität, 8000 München, FRG
Edelman, G.M.	Rockefeller University, New York, NY 1021, USA
Eichmann, K.	Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, 6900 Heidelberg, FRG Institut für Genetik, Universität Köln, 5000 Köln, FRG

Eisen, H.N.	Center for Cancer Research, Harvard-M.I.T. Program for Health Science and Technology, Cambridge, MA 02139, USA
Fischer, H.	Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg, FRG
Günther, E.	Lehrstuhl für Immunbiologie der Universität und Max-Planck-Institut für Immunbiologie, 7800 Freiburg, FRG
Hämmerling, G.	Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, 6900 Heidelberg, FRG Institut für Genetik, Universität Köln, 5000 Köln, FRG
Hamlyn, P.H.	MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England
Henning, R.	Abt. Biochemie II, Universität Ulm, Oberer Eselsberg, 7900 Ulm, FRG
Hess, M.	Max-Planck-Institut für Biochemie, 8033 Martinsried, FRG
Hood, L.	Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA
Hozumi, N.	Basel Institute of Immunology, Grenzacherstrasse 487, 4058 Basel, Switzerland
Huber, R.	Max-Planck-Institut für Biochemie, 8033 Martinsried, FRG Physikalisch-Chemisches Institut der Technischen Universität, 8000 München, FRG
Humphreys, R.E.	Harvard University, The Biological Lab., 16 Divinity Avenue, Cambridge, MA 02138, USA The National Cancer Institute, National Institutes of Health, Bethesda, MD 20014, USA
Jerne, N.K.	Basel Institute of Immunology, Grenzacherstrasse 487, 4058 Basel, Switzerland
Kabat, E.A.	Departments of Microbiology, Human Genetics and Development, and Neurology, Columbia University, The Neurological Institute, Presbyterian Hospital, New York, N.Y. 10032, USA The National Cancer Institute, National Institutes of Health, Bethesda, MD 20014, USA
Kaufman, J.F.	Harvard University, The Biological Lab., 16 Divinity Avenue, Cambridge, MA 02138, USA The National Cancer Institute, Immunology Branch, National Institutes of Health, Bethesda, MD 20014, USA
Mann, D.L.	Harvard University, The Biological Lab., 16 Divinity Avenue, Cambridge, MA 02138, USA The National Cancer Institute, Immunology Branch, National Institutes of Health, Bethesda, MD 20014, USA

Matsushima, M.	Max-Planck-Institut für Biochemie, 8033 Martinsried, FRG Physikalisch-Chemisches Institut der Technischen Universität, 8000 München, FRG
Milner, R.J.	Rockefeller University, New York, N.Y. 1021, USA
Milstein, C.	MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England
Möller, G.	Division of Immunbiology, Karolinska Institute, Wallenberglaboratory, Lilla Freskati, 104 05 Stockholm 50, Sweden
Palm, W.	Institut für Medizinische Biochemie der Universität Graz, Graz, Austria
Parham, P.	Harvard University, The Biological Lab., 16 Divinity Avenue, Cambridge, MA 02138, USA The National Cancer Institute, Immunology Branch, National Institutes of Health, Bethesda, MD 20014, USA
Du Pasquier, L.	Basel Institute of Immunology, Grenzacherstrasse 487, 4058 Basel, Switzerland
Pecht, I.	Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel
Pernis, B.	Basel Institute of Immunology, Grenzacherstrasse 487, 4058 Basel, Switzerland
Phizackerley, R.P.	Dept. of Biophysics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
Poljak, R.J.	Dept. of Biophysics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
Porter, R.R.	Dept. of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QV, England
Potter, M.	Laboratory of Cell Biology, National Cancer Institute, DPHEW, Bethesda, MD 20014, USA
Proudfoot, N.J.	MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England
Rabbitts, T.H.	MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England
Rajewsky, K.	Institut für Genetik, Universität Köln, 5000 Köln, FRG
Reske, K.	Rockefeller University, New York, N.Y. 1021, USA
Robb, R.	Harvard University, The Biological Lab., 16 Divinity Avenue, Cambridge, MA 02139, USA The National Cancer Institute, Immunology Branch, National Institutes of Health, Bethesda, MD 20014, USA

Rüde, E.	Lehrstuhl für Immunbiologie der Universität und Max-Planck-Institut für Immunbiologie, 7800 Freiburg, FRG
Saul, F.	Department of Biophysics, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205, USA
Schrader, J.W.	Rockefeller University, New York, N.Y. 1021, USA
Schuler, W.	Fachbereich Biologie, Universität Konstanz, 7750 Konstanz, FRG
Silver, J.	Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA
Springer, T.	Harvard University, The Biological Lab., 16 Divinity Avenue, Cambridge, MA 021138, USA The National Cancer Institute, Immunology Branch, National Institutes of Health, Bethesda, MD 20014, USA
Strominger, J.L.	Harvard University, The Biological Lab., 16 Divinity Avenue, Cambridge, MA 021138, USA The National Cancer Institute, Immunology Branch, National Institutes of Health, Bethesda, MD 20014, USA
Terhorst, C.	Harvard University, The Biological Lab., 16 Divinity Avenue, Cambridge, MA 021138, USA The National Cancer Institute, Immunology Branch, National Institutes of Health, Bethesda, MD 20014, USA
Tonegawa, S.	Basel Institute of Immunology, Grenzacherstrasse 487, 4058 Basel, Switzerland
Weiler, E.	Fachbereich Biologie, Universität Konstanz, 7750 Konstanz, FRG
Weiler, I.J.	Fachbereich Biologie, Universität Konstanz, 7750 Konstanz, FRG
Wigzell, H.	Dept. of Immunology, Uppsala University, Biomedical Center, Box 582, 751 23 Uppsala, Sweden
Ysern, X.	Dept. of Biophysics, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205, USA
Ziffer, J.A.	Rockefeller University, New York, NY 1021, USA

Contents

The Major Histocompatibility Complex and Cellular Recognition

General Aspects of the Immune System

Antibody Structure

Antibody Structure

The Structural Basis of Antibody Specificity

E. A. Kabat

The extraordinary capacity of the antibody-forming mechanism to make combining sites complementary to almost any molecular conformation of matter ranging in length from about 5Å to about 34Å (Kabat, 1976; Goodman, 1975) must ultimately be explained in structural, biosynthetic and genetic terms. While high resolution X-ray crystallography has now elucidated the structures of a number of combining sites including two Fab (Poljak et al., 1973; Segal et al., 1974), one light chain dimer (Schiffer et al., 1973) and an Fv fragment (Epp et al., 1974; see Davies and Padlan, 1976), it will manifestly be impossible to establish by this method the structures of a sufficiently large number of antibody combining sites of various specificities for us to map in detail the fitting of various antigenic determinants with their complementary regions in all antibody combining sites. Recent estimates suggest 10^7 clonotypes (Klinman et al., 1976) of antibody forming cells.

One must thus endeavor to learn the principles on which antibody complementarity is based by developing predictive techniques which if successful might provide a key to elucidating the structure not of a single specific antibody combining site but of a whole group or class of sites. One could then use such new insight for making and testing additional predictions and ultimately discover the basic principles involved.

Among the unique features of antibody molecules which distinguish them from all other proteins is the existence of a variable region. Recognition of this variable region was made possible by the availability (cf. Frisch, 1967; Killander, 1967; Kochwa and Kunkel, 1971) of myeloma globulins and Waldenström macroglobulins from humans and from mice (Potter, 1971) and the finding (Edelman and Gally, 1962) that Bence Jones proteins, useful for over a century in the diagnosis of multiple myeloma, were the light chains of immunoglobulins. It had been known for many years that the urinary Bence Jones proteins differed in their properties from one myeloma patient to another in physicochemical and immunological (Bayne-Jones and Wilson, 1922) properties, and later the myeloma globulins were also shown to differ in physicochemical and immunochemical properties from one myeloma patient to another (see Gutman, 1948; Killander, 1967; Frisch, 1967; Kochwa and Kunkel, 1971; Nisonoff et al., 1975; Kabat, 1976).

When the first sequence data from two Bence Jones proteins became available (Hilschmann and Craig, 1965), it became clear that the molecule could be divided into two regions, a variable region and a constant region. The variable region was so named because it differed in sequence from one Bence Jones protein to another, while the constant region was uniform for each class of Bence Jones protein. Bence Jones proteins had been divided into two classes immunologically (Bayne-Jones and Wilson, 1922; Korngold and Lipari, 1956) and now termed κ and λ. Myeloma globulin and antibodies produced by the usual immunization procedures were shown to have a basic structural similarity, being built up of monomers or oligomers of a basic four chain structure consisting of two identical heavy and two identical light chains. The studies of Porter (1959) and Nisonoff et al. (1959) led

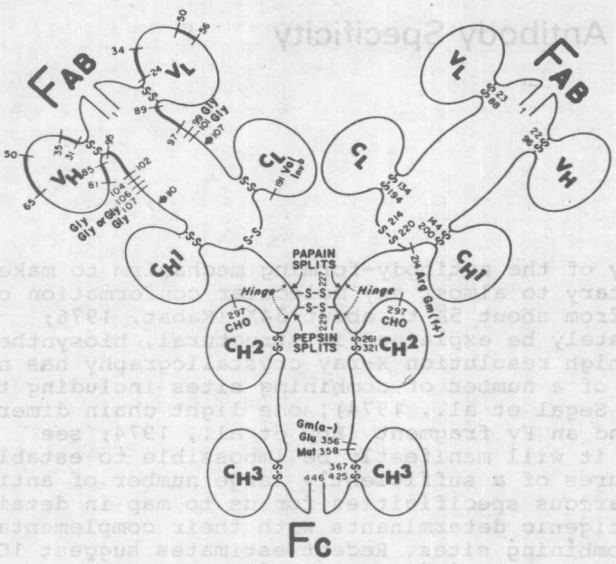

Fig. 1. Schematic view of 4-chain structure of human IgGκ molecule. Numbers on right side: actual residue numbers in protein Eu; Numbers of Fab fragment on left side aligned for maximum homology; light chains numbered as in Wu and Kabat (1970), and Kabat and Wu (1971). Heavy chains of Eu have residue 52A, 3 residues 82A, B, C and lack residues termed 100 A,B,C,D,E and 35 A,B. Thus residue 110 (end of variable region) is 114 in actual sequence. Hypervariable regions: heavier lines. V_L and V_H: light and heavy chain variable region; C_H1, C_H2 and C_H3: domains of constant region of heavy chain; C_L: constant region of light chain. Hinge region in which 2 heavy chains are linked by disulfide bonds is indicated approximately. Attachment of carbohydrate is at residue 297. Arrows at residues 107 and 110 denote transition from variable to constant regions. Sites of action of papain and pepsin and locations of a number of genetic factors are given. (From Kabat, 1973)

to insight into the action of papain and of pepsin in cleaving the molecule into the Fab or Fab' and Fc fragments (Fig. 1), and as the disulfide bonding patterns became known from sequence data, the molecules were seen to have domains each consisting of a disulfide loop of about 65-70 residues and a connecting portion, and each domain was associated with a different function (Fig. 1). The variable region of the light chain and a similar variable region of the heavy chain interacted noncovalently to form a three-dimensional structure with an intact antibody combining site. If light and heavy chains of myeloma globulins or antibodies were separated chromatographically and recombined, an intact combining site would be formed. There also was some degree of specificity of recognition of one heavy chain for its own light chain when given a choice by being presented with a mixture of its own and another light chain (Grey and Mannik, 1965). Another important development was the recognition that some human and mouse myeloma proteins could possess antibody activity, and a host of different specificities have been recognized. To date the crystallographic studies have all been on myeloma proteins and on Bence Jones proteins or their fragments.

While important properties of antibodies are associated with the constant domains, these are in general no different in their genetic control, biosynthesis and evolution from other proteins, so that the discussion of the structural basis of antibody specificity will concentrate on the variable regions of the light (V_L) and heavy (V_H) chains.

If one examines a large number of proteins each having a specific receptor site for a given ligand (such as the cytochromes c of different species) and for which a three dimensional structure has been established by X-ray crystallography, it is very easy to recognize two types of amino acid residues: those making contact with the ligand and forming the specific binding site, and those which are involved only in three-dimensional folding i.e. those which play essentially a structural role in permitting the molecule to assume the proper three-dimensional form. For instance, there is among the cytochromes of various species a preservation of secondary structure which permits the combining site to be in the same place and to function (Takano et al., 1973). The sequence differences among cytochromes of various species thus represent what I have called mutational noise; that is, the permissible mutations, occurring during speciation and evolution, are only selected provided they permit a functioning combining site to form; other mutations, if the receptor site must perform some unique and vital function, would be lethal. It may of course be that some of the mutations confer some additional advantage to each species and have thus been selected and preserved, but this is not relevant to this analysis.

As the number of variable regions of human and mouse Bence Jones proteins and of light and heavy chains of myeloma proteins sequenced began to increase, it became clear that the variable regions could be divided into subgroups (Hood et al., 1967; Milstein, 1967; Niall and Edman, 1967), the number varying with the species, and also that certain positions accommodated many more amino acid substitutions, e.g. showed more variability than others (Milstein, 1967; Kabat, 1968, 1970).

The problem remained of trying to locate and distinguish within the variable region those residues involved in three-dimensional folding from those residues making contact with the antigenic determinant. Reasoning that if one had to generate tens of thousands or even millions of different antibody combining sites, each having a similar three-dimensional structure, Dr. T.T. Wu and I assumed that one should find segments of high variability superimposed upon the usual mutational noise associated with species differences. Having compiled a data bank of the then available sequences of variable regions of human κ, human λ and mouse κ chains aligned for maximum homology, we defined a parameter termed variability (Wu and Kabat, 1970) as follows:

$$\text{Variability} = \frac{\text{number of different amino acids at any position}}{\text{frequency of the most common amino acid at that position}}$$

If at any position in the aligned sequences only one amino acid occurs, e.g. the position is invariant, the numerator and the denominator would both be one and the variability would be one, the lowest possible value. If all 20 amino acids were found at a given position the numerator would be 20, and if they all occurred with equal frequency the denominator would be 1/20 or 0.05 and the variability would be 20/0.05 = 400, the highest possible value. For instance at position 94 in the original data, eight and possibly nine amino acids were found, Ile, Leu, Val, Met, Ala, Arg, Ser, Asp and Asx, a total of 21 sequences had been reported and of these Ser occurred 8 times to give a frequency of 8/21 or 0.38. The variability was thus 8/0.38 or possibly 9/0.38 to give 21.1 or 23.6. When variability so determined was plotted against position, three segments of hypervariability were seen (Fig. 2) and these were postulated to be the complementarity-determining segments which form the antibody combining site. Gaps, considered to be insertion or deletions, were localized to these hypervariable segments (Wu and Kabat, 1970).