RECENT ADVANCES IN IMMUNOLOGY

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PREFACE

The aim of this publication is to present the up-to-date views of the many eminent immunologists who contributed to the scientific program of the 5th European Immunology Meeting held in Istanbul in June 1982. Recent Advances in Immunology is intended for immunologists both in the basic sciences and in clinical medicine. It provides under one cover an assemblage of information about fundamental problems in immunology and clinical applications.

The book opens with Prof. E.A. Kabat's review of the problems in understanding the structural basis of antibody complementarity. The succeeding four papers deal with the role of macrophages in the various stages of immune phenomena. The first of the two articles on T cells reports a product necessary for suppressor activity and the second describes an analysis of precursors of cytotoxic T lymphocytes. The articles dealing with immunogenetics start with the description of new loci in HLA by Prof. J.J. van Rood and co-workers followed by a paper describing the molecular cloning of H-2 class I genes. Prof. P.J. Lachmann begins the discussion on the genetics of the complement system. There are three stimulating articles on the chemistry and genetics of the complement components and their associations with disease. After a review of artificial antigens and synthetic vaccines, papers on immunomodulation describe strategies for improving immunogenicity, immunomodulation in tumor systems and by xenobiotics.

A report on the technical difficulties and improvements of the human-human hybridoma system links the first part of the book with the clinical immunology papers. The discussion of lymphocyte dysfunctions associated with enzyme defects is followed by three papers dealing with various forms of immunodeficiency. The section on autoimmunity starts with the discussion of T cell regulation in autoimmune diseases. A comprehensive paper by Prof. D. Doniach and G.F. Bottazzo describes the principles of early detection of autoimmune endocrine disorders. The role of autoimmune T lymphocytes in the pathogenesis of myasthenia gravis gives a good example of the cooperation between laboratory and clinical medicine. Finally some of the patho-physiological mechanisms of immediate type hypersensitivity

are discussed in three papers: the properties and function of human IgG short term sensitizing anaphylactic antibody, leukotrienes and lipid factors and the regulation of imflammatory reactions by calmodulin.

The contributors deserve all the credit that the meeting may have achieved in bringing into perspective much new knowledge in the various fields of immunology. Their cooperative efforts are much appreciated. We are convinced that a book giving the highlights of the Istanbul Meeting is particulary timely and we hope that it will be well-received.

Asuman Ü. Müftüoğlu, M.D.
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CONTENTS

PROBLEMS IN UNDERSTANDING THE GENERATION OF ANTIBODY COMPLEMENTARITY

Elvin A. Kabat

The National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, and the Departments of Microbiology, Human Genetics and Development, and Neurology, and the Cancer Center/Institute for Cancer Research, Columbia University College of Physicians and Surgeons, New York, U.S.A.

A major problem of the present decade is the elucidation of the structural basis of antibody complementarity, or, in other words, what do antibody combining sites of a given specificity look like and how do the various amino acid side chains make for different kinds of combining sites (1-3). Intimately related to this is the question of how the capacity to make these sites is maintained in the germ line. The genetic basis for the formation of antibody combining sites differs from that of all other proteins with specific receptor sites such as enzymes, hormones and lectins in that antibody combining sites are formed by two chains whereas other receptor sites are essentially built of a single chain. It was generally estimated that mammals could form about 10^6 antibody combining sites but since the development of the hybridoma technique (4) this estimate must clearly be low by several orders of magnitude since to date no two hybridomas making antibody combining sites to a single antigenic determinant such as $\alpha 1 \rightarrow 6$ dextran have been found to be identical (5-7).

Let us consider what we know about the structure of antibody combining sites. As the amino acid sequences of light and of heavy chains of immunoglobulins were being determined, it became clear that both the light and heavy chains had a domain structure each domain having a disulfide bond with a loop of about 55-70 amino acids and that the antibody combining site was associated with the N-terminal domain of each chain termed the V_L and V_H domains (V=variable) respectively (Figure 1). The earliest sequence studies were carried out on human Bence Jones proteins shown by Edelman and

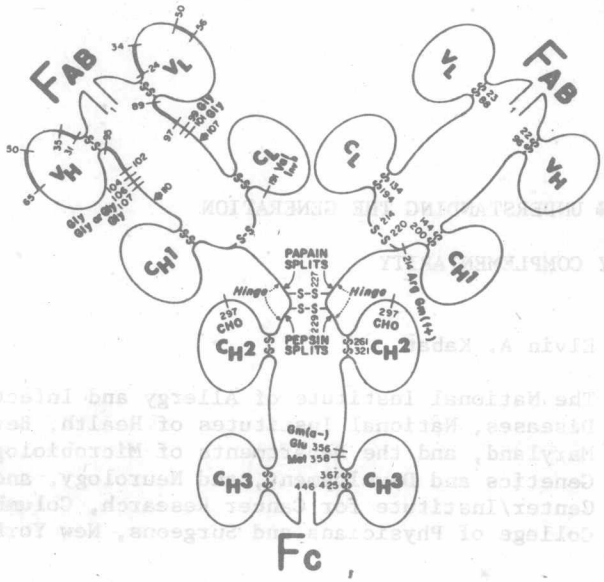

Fig. 1. Schematic view of four-chain structure of human IgG_K molecule. Numbers on right side denote actual residues of protein Eu (8,9). Numbers of Fab fragments on the left side are aligned for maximum homology; light chains are numbered according to Wu and Kabat (10,11). Heavy chains of Eu have residue 52A and 82A, B, C and lack residues termed 100A, B, C, D, E, F, G, H and 35A, B. Thus residue 100 (end of variable region) is 114 in actual sequence. Hypervariable regions and complementarity-determining segments or regions are shown by heavier lines. V_L and V_H denote light- and heavy-chain variable regions; C_H1, C_H2, and C_H3 are domains of constant region of heavy chain; C_L is constant region of light chain. The hinge region in which 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 transition from variable to constant regions. Sites of action of papain and pepsin and locations of a number of genetic factors are given. (Reproduced with the kind permission of Academic Press).

Gally (11) to be the light chains of immunoglobulin; no two human Bence Jones proteins were found to have identical amino acid sequences in their amino terminal first domains and this led to their being termed variable regions. The antigenic specificity of Bence Jones proteins had permitted them to be classified into two classes, κ and λ and these were ascribable predominantly to amino acid differences in the second or C domain (Figure 1); all κ light chains,

had the same amino acid sequences except for two positions at which Mendelian allelism was shown to occur. The three C-domains of the heavy chain were comparable to the C-domain of the light chain and C-domains were responsible for functional properties of antibodies other than their ability to combine with antigen (13).

As amino acid sequences of light chains accumulated, it was possible by aligning the chains for maximum homology to undertake a statistical analysis of each position in the variable region, using the formula (10)

$$\text{Variability} = \frac{\text{Number of different amino acids occurring at any given position}}{\text{Frequency of the most common amino acid at that position}}$$

to show that there were three regions of high variability, termed hypervariable regions (10) and shown by the heavy portions of the V-domain in Figure 1. It was predicted that the chain would fold so that the combining site would be formed by the three hypervariable regions. Mouse V_λ chains were also sequenced; 12 mouse V_λ chains were identical throughout the V-region and seven variants differed by from one to three amino acids all involving one base change and all but one in hypervariable regions. These were ascribed to somatic mutation (14,15). Similar data supporting somatic mutation were found for some mouse V_K sequences (15). When a sufficient number of heavy chain sequences became available, they too were found to have three hypervariable regions (Figure 2) (11). Hypervariable regions are unique to immunoglobulins and are seen in no other collection of proteins such as the cytochromes etc. (1-3). The inference that the combining site was formed by the three hypervariable regions of each was supported by affinity labeling studies (17) and was completely verified when X-ray crystallographic studies of Fab fragments (18, 19) and of Bence Jones protein dimers or Fv dimers were carried out (20-22) and the hypervariable regions in each instance formed the walls of the combining site (Figure 3); thus these are now termed CDR (complementarity-determining regions) (1), the remainder of the domain constituting a framework (FR) which serves to position the CDR at the tip of the molecule. That the FR of the various proteins are essentially similar has permitted attempts to construct antibody combining sites by replacing the CDR of these structures with the CDR of known antibody molecules. A major limitation of the X-ray crystallographic studies to date is that the determinant for which the site is specific is not known, the two Fab fragments (18,19) being from myeloma proteins and the Bence Jones dimers forming primitive sites in which one V_L region orients like the V_H region (20).

Until X-ray crystallographic studies at high resolution are car-

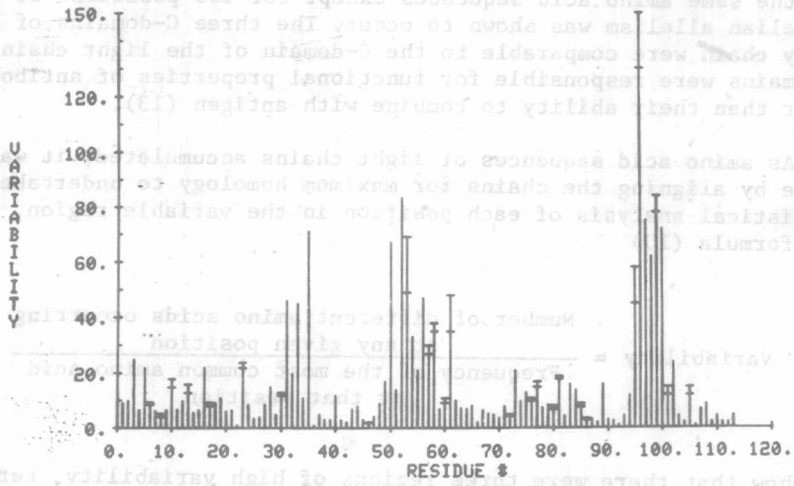

Fig. 2. Variability at different positions for the variable region of heavy chains. The plot was made by the PROPHET computer system (16). (From 37).

ried out on an antibody molecule with a combining site specific for at least one antigenic determinant of defined specificity which fills the site completely and to see how the determinant is oriented in the site, progress in understanding antibody complementarity will be limited. With several or even one such structure rapid strides can be made.

Antibody combining sites may be mapped immunochemically by determining the structure of the hapten which fits into an antibody combining site (23). This is accomplished by a variety of methods which measure the strength of binding directly as an association constant or by measuring the relative capacity of various haptens on a molar basis to displace the antigenic determinant from the site

The reaction between α1→6 dextran and human antidextran provided the first system used to measure the size of an antibody combining site. The isomaltose oligosaccharides (α1→6 linked) from the disaccharide to the heptasaccharide were used to inhibit the precipitin reaction between α1→6 dextran and antidextran and essentially provided a molecular ruler for measuring sizes of antigenic determinants and antibody combining sites (24-26, see 23). On a molar basis the best inhibitors were found to be the hexa-and heptasaccharides. With most antisera, the hepta- and hexasaccharides were equally potent but with antidextran from one individual, the hepta- was

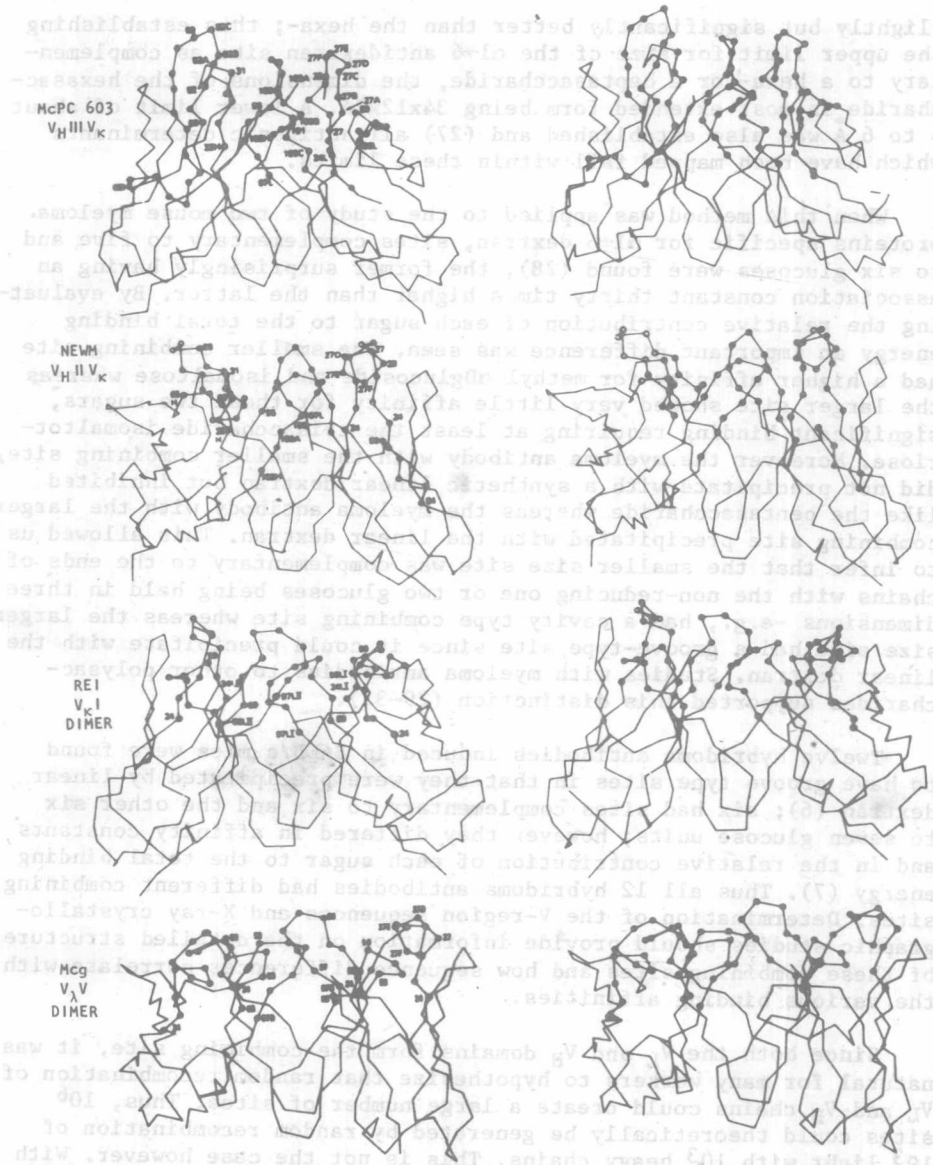

Fig. 3. Stereodrawings of the α-carbon skeletons of the V-regions of four of the five proteins studied crystallographically. Each protein is in the same orientation. With a stereoviewer it is possible to see two adjacent models at the same time so that a comparison may be made in three dimensions.

slightly but significantly better than the hexa-; thus establishing the upper limit for size of the α1→6 antidextran site as complementary to a hexa- or a heptasaccharide, the dimensions of the hexasaccharide in most extended form being 34x12x7Å. A lower limit of about 4 to 6 Å was also established and (27) all antigenic determinants which have been mapped fall within these limits.

When this method was applied to the study of two mouse myeloma proteins specific for α1→6 dextran, sites complementary to five and to six glucoses were found (28), the former surprisingly having an association constant thirty times higher than the latter. By evaluating the relative contribution of each sugar to the total binding energy an important difference was seen. The smaller combining site had a higher affinity for methyl αDglucoside and isomaltose whereas the larger site showed very little affinity for these two sugars, significant binding requiring at least the trisaccharide isomaltotriose. Moreover the myeloma antibody with the smaller combining site did not precipitate with a synthetic linear dextran but inhibited like the pentasaccharide whereas the myeloma antibody with the larger combining site precipitated with the linear dextran. This allowed us to infer that the smaller size site was complementary to the ends of chains with the non-reducing one or two glucoses being held in three dimensions -e.g., had a cavity type combining site whereas the larger size site had a groove-type site since it could precipitate with the linear dextran. Studies with myeloma antibodies to other polysaccharides supported this distinction (29-31).

Twelve hybridoma antibodies induced in BALB/c mice were found to have groove type sites in that they were precipitated by linear dextran (6); six had sites complementary to six and the other six to seven glucose units; however they differed in affinity constants and in the relative contribution of each sugar to the total binding energy (7). Thus all 12 hybridoma antibodies had different combining sites. Determination of the V-region sequences and X-ray crystallographic studies should provide information on the detailed structure of these combining sites and how sequence differences correlate with the various binding affinities.

Since both the V_L and V_H domains form the combining site, it was natural for many workers to hypothesize that random recombination of V_L and V_H chains could create a large number of sites. Thus, 10^6 sites could theoretically be generated by random recombination of 10^3 light with 10^3 heavy chains. This is not the case however. With the 12 α1→6 hybridoma antidextrans which were obtained from a myeloma parent which secreted κ chains, it could be shown that the κ chains from the myeloma parent associated with the heavy chain less well than did the κ chain from the antibody synthesizing cell (5). In the most definitive study with six hybridomas secreting anti-fluorescyl antibody only the six homologous V_L-V_H recombinations were found to bind fluorescein (32). Thus a major problem becomes to learn what

determines association of a given V_L with V_H in the formation of antibody combining sites. To date no one has found a heterologous recombinant which presents a new defined specificity.

In a collaborative study (33) of 12 hybridoma antibodies to dextran B1355S, a highly branched dextran built of alternating α1→3 and α1→6 linked glucoses, it was possible to classify the antibodies into five groups by quantitative precipitin curves with a panel of 20 dextrans. All of the hybridoma antibodies and two myeloma antibodies J558 and MOPC104E reacted best with three highly branched dextrans containing the highest proportion of α1→3 linkages and the alternating α1→6 α1→3 pattern. The classification into the five groups was based on the number of cross reacting dextrans and the extent of cross reactivity. Group 1 reacted only with the three highly branched dextrans and did not react with the other dextrans. One would have expected all three to have identical sequences in the CDR. However, the heavy chains differed in all but one residue in CDR3. This reveals an unsuspected type of antibody specificity which may further complicate precise understanding of the generation of antibody complementarity. If the cross reactions involve a subsite of the antibody combining site, then it would appear that in the group 1 hybridomas, access to this subsite may be blocked by different amino acid side chains in the CDR; an analogous situation has been found in the sites of chymotrypsin as compared with elastase. The former site contains two glycines whereas the latter has valine and threonine which do not permit access of certain chymotrypsin substrates with bulky side chains (34). Another suprising finding is that members of different groups may differ only by a single amino acid in the entire V_H domain. This suggests that the light chains, although λ are contributing to the site complementarity. There also appears to be some correlation between precipitin group, the D region positions 96 and 97 (CDR3) and the MOPC104E individual idiotype (IdI), the two hybridomas and MOPC104E having Tyr and Asp at these positions being in precipitin group 5. Hybridomas with IdI(J558) with Arg and Tyr at these positions and J558 are in groups 1 and 3.

If we now consider the remarkable insights which have come from application of recombinant DNA technology we find that a major change in thinking has been the result of the discovery of intervening sequences and the splicing out of the primary transcript formed in the nucleus to give the cytoplasmic mRNA which is translated on the ribosome to give the protein chain (35). Immunoglobulin genes show two unique features: 1. The variable region is assembled from gene segments during differentiation and 2. the joining of the V to the C region and the switch from one immunoglobulin class to another is accomplished by splicing out not only of the intervening sequences but also of the coding sequences between the chain initially synthesized, IgM, and the chain resulting from the switch. The order of the C-region genes in the mouse has been shown to be 5'-Cμ-Cδ-

$C_\gamma 3$-$C_\gamma 1$-$C_\gamma 2b$-$C_\gamma 2a$-$C\varepsilon$-$C\alpha$-3' (36). We shall only consider the genes or gene segments involved in the synthesis of the V_L and V_H domains.

In organizing our data base of amino acid sequences in the PROPHET computer (37), we noted that two human V_λ chains, a V_λII and V_λV had 21 differences in amino acid composition and yet had an identical first CDR of 14 amino acids (38). A human V_KI and a V_KIII had 30 amino acid differences but were identical in CDR3 (39). As there were very few identical CDR, we ordered the FR segments into sets, all members of a given set having an identical amino acid sequence. When each chain was traced from one FR to another (Figure 4) (40), there appeared to be an independent assortment of FR segments. Moreover, FR4 seemed to be independent of the V_K subgroup, since V_KI could be in the same FR4 set with V_KII, V_KIII, or V_KIV confirming earlier observations that V_K subgroups could not be traced beyond amino acid 94 (41). Similar assortment of FR segments of human and mouse V_HIII chains were seen and it was subsequently possible to assort the FR and CDR segments of rabbit light chains (42).

The first sequence of a clone containing a germ line V_λ gene from 12 day mouse embryo had just been reported (43) and it was found to code only through amino acid 96. It was known that the V-region of the secreted adult plasmacytoma light chain extended through amino acid 107 or 108 and from this and the assortment data, it was hypothesized that the FR and CDR were gene segments (minigenes) and that the genes coding for the FR and by implication for the CDR were assembled somatically during differentiation to give an intact V-region. Shortly thereafter two additional clones were found, one from 12 day old embryo coding for amino acids 97-108, termed the J segment, followed by an intervening sequence and the gene coding for the entire C_L region (44). The second from adult plasmacytoma coded for the intact V-region. Thus the assortment data were confirmed; the nucleotides coding for the J segment, with intervening sequences 5' and 3', constituted a minigene. Cloning of embryo mouse V_K chains, showed that there were five J segments (45,46), one of which J3 was a pseudogene, each separated by intervening sequences. Each coded for amino acids 96-108. The intervening sequence after J5 was followed by the C_K coding region. The four functional J_K minigenes were seen to assort independently (47). Sequences of mouse germ-line genes coding for heavy chains showed the presence of four J_H minigenes (48,49) which also assort independently as seen in the five classes of B1355 antidextran hybridomas (33).

Assortment of FR segments of mouse V_K light chains could also be demonstrated at the DNA level (50). A library from mouse myelomas MOPC21 was screened and 28 V-containing clones were isolated which did not hybridize to C or J region probes and were considered to represent unrearranged embryonic V-region genes. These were used in dot blot screening with probes approximately containing nucleotide sequences coding for the different FR segments. All clones hybridized

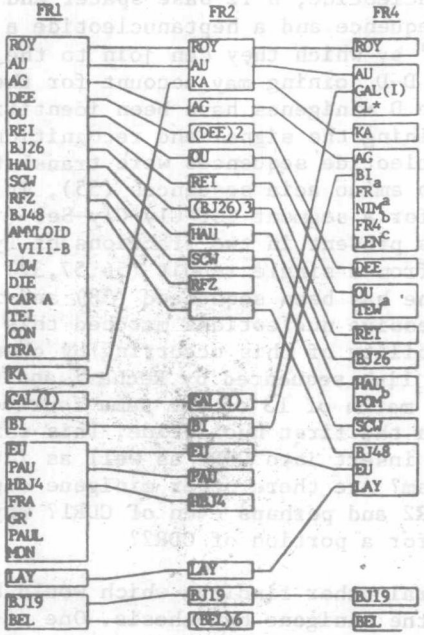

Fig. 4. Independent assortment of framework sets in human $V_K I$ chains. Solid circle, cold agglutinin with antiblood group I activity; a, human κ light chain subgroup II; b, human κ light chain subgroup III; c, human κ light chain subgroup IV. (From 40).

to the intact V-region probe. Six hybridized to all probes for FR1, FR2 and FR3 and probably represent the gene family usually seen by Southern blotting which is apparently less sensitive than dot blotting. Three clones hybridized only to FR1, 10 only to FR2, and 9 only to FR3. While these assortment data are consistent with the minigene hypothesis, sequencing of the clones will be necessary to establish whether or not they are already assembled in the germ line or whether they exist as minigene segments.

When germ line heavy chain genes were sequenced it was noted that the V-region was coded for only through FR3 (48,49). The four J segments generally contained nucleotides coding for FR4 plus several residues of CDR3. However the V and J segments, unlike findings with light chains did not code for the intact chain, segments coding for from five to 14 amino acids were missing. This segment was termed the D or diversity segment and the D segments are also minigenes since they are surrounded by intervening sequences. D minigenes were subsequently isolated (51-53) and found to have signal