ADVANCES IN

Immunology

VOLUME 20

ADVANCES IN
Immunology

EDITED BY

F. J. DIXON
Division of Experimental Pathology
Scripps Clinic and Research Foundation
La Jolla, California

HENRY G. KUNKEL
The Rockefeller University
New York, New York

VOLUME 20

1975

ACADEMIC PRESS New York San Francisco London
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 61-17057

ISBN 0-12-022420-8

PRINTED IN THE UNITED STATES OF AMERICA

LIST OF CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

J. DONALD CAPRA,* *Department of Microbiology, The Mount Sinai School of Medicine, New York, New York* (1)

ALFRED J. CROWLE, *Webb-Waring Lung Institute and Department of Microbiology, University of Colorado School of Medicine, Denver, Colorado* (197)

CHELLA S. DAVID, *Department of Human Genetics, University of Michigan, Ann Arbor, Michigan* (125)

J. MICHAEL KEHOE, *Department of Microbiology, The Mount Sinai School of Medicine, New York, New York* (1)

MARIAN ELLIOTT KOSHLAND, *Department of Bacteriology and Immunology, University of California, Berkeley, California* (41)

MORRIS REICHLIN, *Departments of Medicine and Biochemistry, State University of New York at Buffalo, Buffalo, New York* (71)

DONALD C. SHREFFLER, *Department of Human Genetics, University of Michigan, Ann Arbor, Michigan* (125)

* Present address: Department of Microbiology, University of Texas, Southwestern Medical School, Dallas, Texas 75235.

PREFACE

Volume 20, like its predecessors, reflects several of the most active and important areas of immunologic research, presenting three reviews relating the structure of antigens or antibodies to their participation in immunologic reactions. A fourth deals with the genetic control of immunologic events and the final review analyzes *in vivo* cellular immune reactions. The specific subjects are the structure of V regions of Ig molecules, the structure and role of J immunoglobulin chain in polymerization and perhaps in control of the immune response, the structural basis and genetic control of the antigenic specificity of globular proteins, an analysis of the *H-2* gene complex with its determination not only of histocompatibility but also of the nature of the immune response, and, finally, the phenomenon of delayed hypersensitivity—the classic expression of cellular immunity.

In the first article, Drs. Capra and Kehoe discuss the structure and role of the variable regions of the Ig molecules. Based on amino acid sequence data, hypervariable regions make up a significant part of the variable regions, occupy relatively constant locations in a variety of Ig molecules even from different species, and appear to be intimately associated with the antibody-combining site. In addition, the idiotypic determinants that mark the antigenic individuality of particular Ig molecules are based on properties of some or all of the hypervariable regions. In contrast, outside the hypervariable regions there is a considerable invariance of sequence, and these invariant sections occur in approximately the same position in Igs of different V region subgroups.

The polymeric Ig molecules are intriguing since one of them, IgM, is structurally the most complex, phylogenetically the most primitive, and ontogenetically the earliest of the Ig molecules. The other polymeric Ig molecule, IgA, evolved relatively recently but retained that portion of the IgM Fc sequences responsible for polymerization and, in addition, developed a mechanism for transport across epithelial cells. Formation of these polymeric molecules involves a distinct third immunoglobulin chain —the J chain—which is the subject of the article by Dr. Koshland. This paper is an authoritative presentation of current knowledge of the role of the J chain in determining the initiation of synthesis, the assembly, and the biologic properties of polymer Ig. It is particularly important since the J chain, via its essential role in IgM polymerization, appears to be a critical factor in the initiation of the immune response and its conversion from IgM to IgG production.

Definition of the basis of antigenicity of globular proteins is com-

plicated by the fact that such molecules contain both sequential determinants, related to amino acid sequence of peptides, and conformational determinants, the product of protein conformation not expressed in linear peptides. In the third article, Dr. Reichlin reviews this subject and emphasizes the role of conformational determinants, deriving much of his data from studies of amino acid substitutions in series of proteins, relating these substitutions to their antigenicity and conformation. The experimental approaches employed to provide the amino acid substitutions are chemical modification of single residues, use of naturally occurring amino acid mutants, and use of a series of genetically homologous proteins of known sequence. These studies indicate that antibodies are best produced to regions of protein antigens bearing sequences different from those of homologous proteins of the immunized specie. Also, globular proteins have highly immunogenic, relatively constant surface patches, the specificity of which is determined by their amino acid composition. Further, these studies indicate the importance of slight genetic changes in determining antigenic specificity, since in certain crucial sites a single amino acid difference resulting from one point mutation can alter conformation and determine complete specificity between two protein antigens.

One of the most rapidly developing areas of immunologic research deals with the *H-2* gene complex, a tightly linked series of genes controlling a variety of immunologic traits including histocompatibility and immune responsiveness. Drs. Shreffler and David, in the fourth paper, draw on their own extensive work in reviewing the remarkable progress in this field. The mapping of the *H-2* complex into four major regions marked by *H-2K, Ir-1, Ss-Slp*, and *H-2D* genes plus the associated *Tla* gene is discussed, along with the phenotypic traits associated with these regions. Particular attention is paid to the immune response region, the genes of which appear to control a variety of immune phenomena including antibody response to many antigens, susceptibility to tumor viruses, GvH and MLC reactions, a system of lymphocyte allo-antigens, and even transplantation antigens. It is becoming apparent that the *H-2* complex consists of many genes with diverse functions, most of which control cell membrane structures and/or processes. The fact that lymphocytes are particularly affected by *H-2* genes has important implications for immunology. However, some of the genes also affect other cell types, implying a still larger role for the *H-2* complex, perhaps in development or in cell regulation. Because the *H-2* complex is the most thoroughly characterized segment of a mammalian chromosome, it is also an important model for studies of gene action, organization, and evolution in mammals.

In the last review, Dr. Crowle discusses in detail delayed-type hyper-

sensitivity, the prototype model of cellular immune phenomena as it occurs in mice. Most of the recent explosive development of the field of cellular immunology has emphasized *in vitro* demonstrations of cellular responses to immunologic challenge and the various products of such stimulated cells which possibly have *in vivo* phlogogenic or cytotoxic potential. However, before the full significance of cellular immune responses in *in vivo* situations can be determined, it will be necessary to transfer the *in vitro* observations to *in vivo* delayed hypersensitivity reactions. For this purpose the mouse appears to be the most likely subject, and Dr. Crowle's review presents both the practical aspects of the various delayed-type hypersensitivity reactions in this species and the probable relationship of these *in vivo* phenomena to the more completely defined *in vitro* reactions. For those who will have to correlate the *in vitro* cellular immune responses to *in vivo* situations, this article will provide a valuable source of background information.

As always, the Editors wish to thank both the authors, who have given generously of their time and meticulous effort, and the publishers, who do much to ensure a volume of high quality.

FRANK J. DIXON
HENRY G. KUNKEL

CONTENTS

Hypervariable Regions, Idiotypy, and the Antibody-Combining Site

J. DONALD CAPRA[1] AND J. MICHAEL KEHOE

Department of Microbiology, The Mount Sinai School of Medicine, New York, New York

I. Introduction

A rational molecular explanation for the capacity of the humoral immune response to interact with an apparently infinite variety of antigenic stimuli seems to be close at hand. The near realization of this long sought

[1] Present address: Department of Microbiology, University of Texas, Southwestern Medical School, Dallas, Texas 75235.

goal of immunochemistry is due to many different kinds of studies from many laboratories. Of prime importance has been the application of the concepts and methodology of contemporary molecular biology to that diverse family of proteins known as immunoglobulins.

In this review, current information relating to antigen binding will be analyzed in the context of these recent developments. Specific attention will be given to the nature and location of hypervariable regions, the idiotypic characteristics of immunoglobulins, and the chemical nature of the combining site itself. The overall aim will be to show the interrelatedness of these various aspects of antibody construction and to forge from these considerations a unified and coherent view of how the humoral immune response fulfills its antigen-binding function.

II. Structure of the Variable Regions of Immunoglobulin Heavy Chains

A. The Twelve Complete Human V_H Sequences

As of this writing, the variable regions of twelve human myeloma proteins lacking known antibody activity have been sequenced. Six of these sequences became available in 1969 and 1970 (Edelman *et al.*, 1969; Cunningham *et al.*, 1969; Wikler *et al.*, 1969; Press and Hogg, 1970; Ponstingl *et al.*, 1970). Six additional sequences have become available recently (Watanabe *et al.*, 1973; Capra and Kehoe, 1974a). All these sequences are displayed in Fig. 1. The proteins have been arranged into V-region subgroups (Cunningham *et al.*, 1969; Kohler *et al.*, 1970; Wang *et al.*, 1971; Capra, 1971; Kehoe and Capra, 1971). The four proteins, Ou, He, Daw, and Cor, clearly are closely related and have been grouped as the V_HII subgroup. Proteins Tie, Was, Jon, Zap, Tur, Nie, and Gal are also closely related and clearly distinct from the above four proteins and have been placed in the V_HIII subgroup. The V_HI subgroup is less clear. Protein Eu shows considerable difference from proteins within the V_HII and V_HIII subgroups. However, since it is the only complete sequence available for the V_HI subgroup, there is some reluctance to assign it to a separate subgroup. However, fragmentary sequence data on proteins Ca (Pitcher and Konigsberg, 1970), Ste (Fisher *et al.*, 1969) and Bro (J. E. Hopper, personal communication) indicate that they are all more related to protein Eu than to proteins of either of the other subgroups. Since protein Eu was the first complete human V_H sequence available, most authors have assigned it to subgroup I.

Fig. 1. The amino acid sequence of the variable regions of twelve human immunoglobulin heavy chains. Protein Ou has a Tyr insertion between 60–61 (marked with an *) and a Tyr-Tyr-Tyr insertion between 109–110 (marked with an ⁂).

A discussion of the twelve human sequences can be conveniently based on those sections of the *V* region that are relatively invariant (1–30, 38–50, 69–83, 92–100, and 111–124) and the hypervariable regions (31–37, 51–68, 84–91, and 101–110).

1. *Relatively Invariant Portion of the V Region of the Heavy Chain*

About 65% of the *V* region of the human heavy chain shows limited sequence variation. In fact, there are twenty-five positions (2, 4, 8, 14, 22, 25, 26, 38, 40, 43, 44, 47, 48, 49, 69, 79, 92, 93, 94, 96, 98, 117, 120, 122, 123), totaling 20% of the *V* region, which are absolutely invariant in the twelve human heavy chains, regardless of their *V*-region subgroup assignment. Certain positions are subgroup-specific since at these positions all, or nearly all, the members of one subgroup have a particular amino acid, whereas members of the other subgroup contain a different residue. Utilizing the four available V_HII proteins and comparing them to the seven V_HIII proteins, positions 3, 9, 17, 19, 21, 23, 28, 29, 39, 42, 46, 50, 70, 80, 81, and 82 appear to be subgroup-specific. As noted previously, no subgroup-specific residues are identifiable in the C-terminal third of the *V* region (Kehoe and Capra, 1971). There are thus forty-one positions (33%) that are either invariant or subgroup-specific.

Within the relatively invariant portion of the *V* region, and within a single subgroup, there is a remarkable similarity between the proteins. Thus, within the V_HII subgroup, if one compares any protein to the subgroup prototype sequence (see below), such a protein will differ from the prototype in only seven positions. In the V_HIII subgroup, most proteins differ from the prototype sequence by only five residues in the relatively invariant portion of the *V* region.

2. *Hypervariable Positions*

Figure 2 depicts the variability of available, human, heavy-chain, *V*-region sequences according to the method of Wu and Kabat (1970). These calculations were based on twenty-five sequences from residues 1–34, twelve sequences from residues 35–85, and fifteen sequences from residues 86–124. Four broad regions of sequence hypervariability are apparent, comprising residues 31–37, 51–68, 84–91, and 101–110. The specific regions of hypervariability differ somewhat within each subgroup. For example, for the second hypervariable region, the section from 51 to 60, is particularly variable in the V_HIII subgroup, whereas in the V_HII subgroup the variability is more marked in positions 61–65.

The extraordinary variability within these hypervariable regions is illustrated best in the V_HIII subgroup. Of the twenty-eight hypervariable positions within this subgroup, a given protein differs from every other

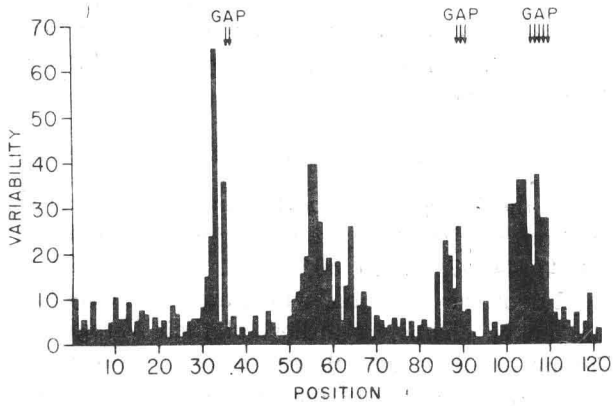

FIG. 2. Variability factor values for the sequences shown in Fig. 1 and several other partial sequences. (The graph is constructed according to the method of Wu and Kabat, 1970.)

protein in twenty-one of these twenty-eight positions. Thus in the relatively invariant portion of the V region, proteins in the V_HIII subgroup are, on the average, identical in 91/95 positions (96%), whereas in the hypervariable regions, they are identical in only 7/28 positions (25%).

3. *V/C Bridge*

A precise localization of the V/C bridge is impossible at this time because of the lack of sequence data in the C_H1 domain for the IgA, IgD,

						120						125			
Eu	(IgG1)	GLY	GLY	LEU	VAL	THR	VAL	SER	SER	ALA	SER	THR	LYS		
Daw	(IgG1)	GLY	ILE	LEU	VAL	THR	VAL	SER	SER	ALA	SER	THR	LYS		
Cor	(IgG1)	GLY	THR	PRO	VAL	THR	VAL	SER	SER	ALA	SER	THR	LYS		
He	(IgG1)	GLY	THR	LYS	VAL	ALA	VAL	SER	SER	ALA	SER	THR	LYS		
Nie	(IgG1)	GLY	THR	LEU	VAL	THR	VAL	SER	SER	ALA	SER	THR	LYS		
Jon	(IgG3)	GLY	THR	PRO	VAL	THR	VAL	SER	SER	ALA	SER	THR	LYS		
Ou	(IgM)	GLY	THR	THR	VAL	THR	VAL	SER	SER	GLY	SER	ALA	SER		
Gal	(IgM)	GLY	THR	LEU	VAL	THR	VAL	SER	THR	GLY	SER	ALA	SER		

FIG. 3. The human heavy-chain V/C bridge. The invariant residues and all residues that are unique to a particular class are boxed.

and IgE myeloma proteins. The available sequences are shown in Fig. 3. The invariant glycine at position 117 (the first invariant residue in the chain after the half-cystine at position 98) is followed by two variable positions to positions 120–121 which are Val-Thr in eight proteins, and Val-Ala in protein He. The eight proteins remain identical to position 124 where a threonine is present in the IgM protein Gal. If the data are correct, and Ou and Gal prove to belong to the same IgM subclass, then the first position that absolutely distinguishes IgG proteins from IgM proteins is position 125 where the IgG myelomas contain alanine and the IgM proteins contain glycine. From this point on they are quite different with IgG proteins having Ala-Ser-Thr-Lys and the IgM proteins showing Gly-Ser-Ala-Ser.

It is of interest that the virtually invariant sequence Thr-Val-Ser-Ser- (121–124), with the exceptions noted above (alanine position 121 in He and threonine position 124 in Gal), is also extremely conserved in animals (see Section II,B). The mouse (MOPC 315; Francis *et.al.*, 1974) and rabbit (Strosberg *et al.*, 1972; Pratt and Mole, 1974) have an identical sequence in the same location.

B. The Animal Heavy-Chain Sequences

To date one mouse myeloma heavy-chain V region has been completely sequenced (Francis *et al.*, 1974), and a second mouse myeloma heavy chain is virtually completed (Bourgois *et al.*, 1972). Although myeloma proteins have been found in other species (Capra and Hurvitz, 1970; Kehoe *et al.*, 1972), these two mouse heavy chains represent the most complete nonhuman sequences. The prototypic sequences of the three human subgroups are displayed in Fig. 4. These prototype sequences are arbitrary in some positions, but, in general, there is little difficulty in constructing such a sequence. Where a position appears variable it is marked with a "V." For the V_HI subgroup, except for the first hypervariable region, no variable positions can be defined since there are so few sequences available at this time.

The pooled guinea pig heavy chains (from strain 13 guinea pig IgG2) have been sequenced by Cebra *et al.* (1971). Certain positions that are variable in the pooled sequence are more restricted in specifically purified antibodies (Cebra *et al.*, 1971; see below). The pooled, rabbit, heavy-chain data are from Wilkinson (1969), Mole *et al.* (1971), and L. E. Mole (personal communication). No alternative amino acids are depicted in positions 2 and 3 of a3 rabbits as originally reported by Wilkinson, since more recent work suggests that this sequence represents the a blank molecules (Prahl *et al.*, 1973). The single rabbit Aa2 sequence is from Fleischman (1973).

1. Subgroup Assignment of Sequenced Animal Immunoglobulin Heavy Chains

It is relatively easy to assign the MOPC 173 sequence and the pooled guinea pig sequence to the V_HIII subgroup. No sequence gaps are required to align these sequences and, excluding the hypervariable positions, there is very strong homology. The MOPC 315 appears more closely related to the human V_HII subgroup, although two sequence gaps are required for maximum homology. The three rabbit sequences are very difficult to relate to the human prototypic sequences but are probably most closely related to the V_HII subgroup.

2. Phylogenetically Associated Residues

As previously noted (Kehoe and Capra, 1972; Capra *et al.*, 1973), it is necessary to search for phylogenetically associated residues within the same subgroup when comparing proteins from different species. In this way a number of such residues are apparent in the heavy-chain sequences from different species (see Kehoe and Capra, 1974, for an extensive review of this subject). However, the observation that there are no positions where all three human subgroup prototypic sequences have a given amino acid residue while the two mouse sequences have a different residue, strongly implies that subgroup divergence occurred prior to speciation.

3. The "Invariant" Positions

There are twenty-four positions (20%) that are invariant in the three human prototypes, the two mouse myeloma sequences, and the rabbit and guinea pig pools. In fact, there are very few positions (2, 4, 8, 14, 26, 30, 69, 79, 120) in which the three human prototypes are identical while the animal sequences are different. Even in such cases, there is generally only one exception (8, 14, 26, 69, 120).

The distribution of the invariant amino acids is of interest since they are grouped into three distinct clusters. Of the twenty-four invariant positions, only four precede the first hypervariable region (4/30 = 13%). Between the first and second hypervariable regions, 8/12 positions are absolutely invariant across the four species (66%), but there are no additional invariant positions until immediately after the third hypervariable region. In this region, from residues 92 to 99 (eight positions), six are

---

FIG. 4. The human prototype sequences for each of the subgroups and the available animal heavy-chain *V* region sequences. Gaps have been introduced to align all the sequences. Only those species where relatively complete data are available have been included. See text for further details.

absolutely invariant (75%). Finally, the last four residues of the variable region, as currently defined, are invariant.

The distribution of these invariant residues, between hypervariable regions 1–2 and 3–4 and at the C terminus of the *V* region could be explained by certain tertiary structural constraints that might be required to maintain the conformation of the *V* region to present the hypervariable regions in the structurally most favorable way for participating in the combining site. This has been previously suggested for the region between 92–100 by Bourgois and Fougereau (1970) and expanded upon for other sections by ourselves (Kehoe and Capra, 1971).

Another possibility may be entertained, however. The particular location of these invariant residues could serve as a recognition unit at the DNA level to insert the hypervariable regions into the backbone *V*-region structural gene, with the C-terminal invariant residues serving in the joining function to the C_H1 domain of the heavy chain. Confirmation or refutation of this idea is not possible at this time.

III. Idiotypy and Cross-Idiotypic Specificity

The antigenic individuality of myeloma proteins was discovered in 1955 (Slater *et al.*, 1955). Eight years later the concept was extended to include certain antibodies (Kunkel *et al.*, 1963; Oudin and Michel, 1963). The structural and genetic implications of these early observations have only been appreciated in the past few years. These workers found that every myeloma protein and/or antibody molecule possesses certain antigenic determinants unique to that protein. The antisera resulting from immunization with a particular protein, after prolonged and exhaustive absorption, still reacted with the immunizing agent. The term *idiotypy* was introduced by Oudin (1966) to designate those antigenic determinants in a population of antibody molecules that are not observed in a population of other immunoglobulins from the same animal nor in antibody directed to the same antigen raised in other animals of the same species. A distinction was thus made by these groups of workers between individual antigenic specificity (obtained operationally by immunization of immunoglobulins from one species into another species; see Kunkel and his co-workers) and idiotypic determinants (obtained operationally by inoculation of the immunoglobulins from one species into other, generally allotypically matched, members of the same species; see Oudin and his co-workers). These types of immunization (based on genetic differences in donors and recipients) are currently referred to as measuring (*1*) heterologous idiotypy (between species), (*2*) homologous idiotypy (within species), and (*3*) isologous idiotypy (with genetically similar animals of the same species) (Potter and Kunkel, 1971).

The idiotypic determinants were soon localized to the Fab fragment of immunoglobulins, and extensive work suggested that, although idiotypic determinants could be localized to either the heavy or the light polypeptide chains, generally both chains contributed to the determinants involved when whole immunoglobulin molecules were used to elicit the idiotypic antisera (Grey *et al.*, 1965). The *V* regions of both heavy and light chains were thus assumed to contribute to these determinants. Nonetheless, a contributory role for the constant portion of the light chain and the C_H1 domain of the heavy chain (Edelman *et al.*, 1969) was not ruled out until Wells *et al.* (1973) demonstrated that the F_v fragment (Inbar *et al.*, 1972) of mouse myeloma MOPC 315 inhibited the reaction between idiotypic antisera and the intact molecule. This F_v fragment, which was the product of an enzymatic cleavage of the entire molecule, was subsequently shown to consist of the *V* regions of both the light and heavy chains, with no constant portion present. These experiments conclusively localized the idiotypic determinants to the *V* regions. An even more precise localization within the *V* region is under current investigation (see Section III,E).

Since this review will focus on the relationships between idiotypy and the antibody-combining site, we will only briefly mention the use of idiotypic determinants by several investigators to study many immunological parameters in novel ways. For example, Wernet *et al.* (1972), using a complement-mediated cytotoxicity assay, demonstrated idiotypic determinants on a significant proportion of the peripheral blood lymphocytes of two individuals with monoclonal serum IgM components. These findings, along with those of others (Preud'homme and Seligmann, 1972) suggest that precursors of cells that actively secrete antibody have on their surface antigenic receptors that are similar (i.e., idiotypically comparable) if not identical to the immunoglobulin that is eventually secreted by them. These investigators thus utilized idiotypic determinants to establish indirectly the identity of immunoglobulin *V* regions.

Eisen and co-workers have used idiotypic determinants as tumor-specific transplantation antigens (Lynch *et al.*, 1972; Hannestad *et al.*, 1972). These experiments open an entirely new approach to tumor immunology and reinforce the notion that the idiotypic determinants are cell-surface markers. In one experiment, Balb/c mice were immunized with MOPC 315 myeloma protein and shown to make antibodies with idiotypic specificity. Subsequently, these mice were transplanted with the same tumors and the tumor growth was found to be suppressed. In some experiments stable variant tumors (producing only light chains) resulted.

Nisonoff and co-workers, in a series of papers (Hart *et al.*, 1972, 1973;