HANDBOOK OF EXPERIMENTAL IMMUNOLOGY
IN FOUR VOLUMES

# Volume 3: Genetics and Molecular Immunology

EDITED BY

D. M. WEIR MD, FRCP

CO-EDITORS

L. A. Herzenberg PhD  Caroline Blackwell PhD

Leonore A. Herzenberg PhD

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# Preface

The pace of progress in immunology has not slackened since the last edition of this handbook. The subject now draws heavily on molecular biology and genetics and this has necessitated the inclusion of an additional volume on **Genetics and Molecular Immunology**. The explosion in the development of hybridoma technology and cell culture, since the last edition, can be seen from the many chapters in each volume that employ monoclonal reagents and cell lines. Some idea of the expansion of the field can be gained from the **Cellular Immunology** volume where contributions on phagocytes and lymphocytes now occupy 30 chapters compared to 12 in the previous edition. A new section on immunoregulation contains 14 chapters and there are now 6 chapters devoted to mammalian cell membrane antigens in the **Immunochemistry** volume.

It is now no longer possible for one editor to keep in touch with the enormous expansion in this field, and I am much indebted to my co-editors Len and Leonore Herzenberg who have joined me in the task of co-opting research workers in the wide range of disciplines now contributing to the field of immunology. I am particularly grateful to my wife Dr Caroline Blackwell for her help with the massive editing task.

Amongst the many new features of this edition is the provision of overviews for many of the sections. I am most grateful to our contributors in the methodology sections for their efforts to achieve a consistent style of presentation of the procedures, and I hope that this will help in the accessibility of the descriptive material. A work of this size inevitably takes a number of years to put together but considerable effort has gone into introducing up to date material into the chapters. This has been achieved by enabling and encouraging contributors to introduce new material and references during the proof stages of their chapters.

I wish to thank Hilary Flenley for her careful and thorough index, and Nigel Palmer and his staff at Blackwell Scientific Publications Edinburgh office without whom production of the new edition would have been impossible. Per Saugman has as always, maintained a benevolent paternal interest in the project.

D.M.W.

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# Chapter 85
# Overview: Introductory comments on molecular immunology

D. BALTIMORE



Molecular immunology is, today, largely the study of the nucleic acids involved in the specification of immunoglobulin structure and concentration. The initial studies of immunoglobulins as proteins, however, posed the questions that were later solved using nucleic acid methodologies. Protein structural analysis taught us to see the immunoglobulin molecule as a hierarchical organization of fragments.

At the grossest level, the immunoglobulin molecule consists of two polypeptide chains, a light chain and a heavy chain. These two chains form a heterodimer that readily associates in pairs to produce the monomeric, 4-chain molecule. At a finer level, each of the two polypeptide chains can be separated into protein domains of approximately 110 amino acids. The most N-terminal of the two domains in the light chains and of the four domains of the heavy chain have a highly variable amino acid sequence. The other domains of the molecules fall into a small number of classes and within any one class the structure is quite constant. Thus protein structural analysis posed the central problem of molecular immunology 'how do you construct a polypeptide with a variable N-terminal structure appended to a chain of basically constant structure?'

Dreyer & Bennett [1] published the most widely held belief about how the unique structure of immunoglobulin polypeptides could have come about. They suggested that two sets of genes are involved: one large library of variable regions and a second very small number of constant regions. A given immunoglobulin molecule would thus have to be made by the recombination of protein molecules, messenger RNA molecules or DNA molecules.

At the finest protein structural level there is a further differentiation of immunoglobulin structure. Wu & Kabat [2], by careful analysis of the primary structure of variable regions, showed that the variable region is actually composed of two types of sequences: hypervariable regions and moderately variable regions. These are interspersed through the variable region giving rise to five mini-segments. Later work has indicated that the hypervariable regions are the parts of the immunoglobulin molecule that directly contact antigen and, therefore, we now call them complementarity-determining residues or CDRs. The seven subregions of the variable region are therefore denoted FR1 (framework 1), CDR1, FR2, CDR2, FR3, CDR3, FR4.

The final level of variation that was identified by sequencing proteins was variation in the structure of the N-terminal domain that arises due to somatic mutation. Here, Weigert *et al.* had the insight to realize that the variable regions of mouse $\lambda$ light chains was probably encoded by a single gene segment [3]. Thus the variation of structure in the N-terminal domain of $\lambda$ molecules must be a consequence of somatic variation of structure rather than of genetic variation. They originally postulated that a somatic mutation mechanism gave rise preferentially to changes in the CDRs.

Analysis of immunoglobulin protein structure thus uncovered three levels of sequence variability that we now recognize as the three fundamental mechanisms leading to immunoglobulin diversity. These mechanisms are: (1) evolution of a large library of different structures; (2) joining of pieces from the libraries to create combinatorial diversity; (3) somatically generated further variation of the inherited immunoglobulin structures. Molecular immunologists working with nucleic acids uncovered the basis of these mechanisms in a much less orderly fashion than they can now be presented. To give honour where it is due, we must first discuss the combinatorial mechanisms and then later consider how evolution generated the large set of variable regions and how somatic mutation is able to further vary them.

**Combinatorial variation**

Immunology moved from the stage of protein structure analysis to that of molecular genetics with the experiment of Hozumi & Tonegawa [4] who showed that synthesis of a $\kappa$ light chain immunoglobulin molecule is accompanied by a reorganization by the DNA that encodes the variable and constant regions. Their experiment implied that the puzzle codified by Dreyer & Bennett had its solution at the DNA level. A flood of further publications from many laboratories then clarified the picture showing us that, for the $\kappa$ light chain, there is a large library of the $V_\kappa$ regions and a single $C_\kappa$ gene segment [5]. Formation of a completed piece of DNA that can encode a $\kappa$ protein involves the joining of one out of the library of $V_\kappa$ segments to the single $C_\kappa$ region. That joining is mediated by a set of five $J_\kappa$ regions located upstream from $C_\kappa$. Examination of the sequence at the joining site between the $V_\kappa$ segments and the $J_\kappa$ segments further showed a slight variability in the position of that join, giving rise to microcombinatorial sequence heterogeneity at the position of the join. The $J_\kappa$ segment, to a first approximation, contributes FR4 to the molecule; the microcombinatorial variation occurs in CDR3 and the rest of the $V_\kappa$ region variability must then be due to evolutionary variation in the sequences of the $V_\kappa$ library.

The situation with the $\lambda$ light chain genes is quite different from that of $\kappa$. In the mouse, there are four $C_\lambda$ regions and only two $V_\lambda$ regions. This apparent violation of our notion of what is variable and what is constant was, like most of the other facts of molecular immunology, presaged by protein structural analysis showing classes of $\lambda$ molecules. This complexity is a minor footnote, however, because the $\lambda$ variable regions, like the $\kappa$ variable regions, are put together by the joining of $V_\lambda$ segments to $J_\lambda$ segments.

For heavy chains the situation is a bit more complicated because there is a tripartite origin of heavy chain variable regions. The segments of the gene are drawn from three libraries: $V_H$ segments, D segments and $J_H$ segments (Fig. 85.1). Two joining reactions are therefore involved, one joining D to $J_H$ and the other joining $V_H$ to D. As with light chains, the $J_H$ segments contribute the FR4 region but the heavy chain CDR3 region is formed by a combination of D-to-$J_H$ and $V_H$-to-D joining. Recent evidence has shown that the D-to-$J_H$ join is made first, followed by $V_H$ joining to the preformed D–$J_H$ segment (Fig. 85.2) [15]. There are four $J_H$ regions, of the order of twenty D segments and probably hundreds of $V_H$ segments; therefore an enormous potential variability exists within the CDR3 region.

There is, however, another sequence element contributing to CDR3 which generates an even greater variability. This was first recognized by a combination of protein and nucleic acid sequence analysis, which showed that CDR3 structure cannot be completely explained by sequences encoded in $V_H$, D and $J_H$. This led to the author's postulate that there is nucleic acid sequence inserted between D and $J_H$ and between $V_H$ and D during the process of joining [6]. It has long been known that T lymphocytes and B lymphocytes that are in the process of developing their specificity contain an enzyme able to synthesize random DNA polymers (terminal deoxynucleotidyl transferase; [7]). These random polymers have a tendency to be high in G residues. Because the sequence elements that are not encoded in the heavy chain libraries also appear to be rich in G residues (or C residues that are complementary to G residues), it was suggested that there are N regions inserted between D and J and between V and D by semi-random polymerization of nucleotides. A recent detailed analysis of the structure of D–$J_H$ and $V_H$–D–$J_H$ regions in developing immunocytes has borne out this prediction of the existence of N regions. Fig. 85.3 shows the sequences of $V_H$ regions as they are forming. In this figure, another element of sequence variation becomes evident: as D and $J_H$ regions are joined, they are often trimmed back at the joining point, thus providing significant variability as a consequence of the specific sequences that appear at the joint. In one case, a D–$J_H$ region has formed and then subsequently been removed as the $V_H$ region is appended because of the trimming process. Because of this trimming, we denote a trimmed region by putting a 'prime' at the side of the region that has been truncated. Thus a complete heavy chain variable

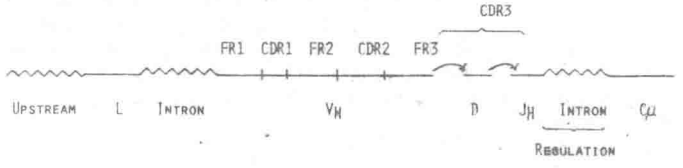


**Fig. 85.1.** Formation of a heavy chain variable region. The three encoded DNA elements that contribute to the heavy chain are the $V_H$ segment (contributing L, the leader, FR1,2,3 and CDR1,2); the D segment (contributing the core of CDR3) and the $J_H$ segment (contributing mainly FR4). The $J_H$ segment is followed by an intron that has crucial regulatory signals and then the constant region of the $\mu$ chain.