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Wu T T & Kabat E A. An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity. *J. Exp. Med.* 132:211-50, 1970; **Kabat E A & Wu T T.** Attempts to locate complementarity-determining residues in the variable positions of light and heavy chains. *Ann. NY Acad. Sci.* 190:382-93, 1971. [Depts. Microbiol., Neurol., and Human Genet. and Develop., Coll. Physicians and Surgeons, Columbia Univ., and Neurol. Inst., Presbyterian Hosp., New York; Depts. Phys. and Engineer. Sci., Northwestern Univ., Evanston, IL; and Biomath. Div., Grad. Sch. Med. Sci., Cornell Univ., and Sloan-Kettering Inst., New York, NY]

These papers made use of available sequence data in 1970 and 1971 of variable regions of light and heavy chains of immunoglobulins to predict that the antibody-combining site was formed by hypervariable regions and that they contained the complementarity determining residues. [The *SCI*® indicates that these papers have been cited in over 630 publications.]

Complementarity-Determining Regions of Antibodies

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When I was at Cornell University Medical College, I read two very interesting articles[1,2] by Elvin A. Kabat of Columbia University College of Physicians and Surgeons analyzing the amino acid distributions of light chains of immunoglobulins. Since I knew very little about immunology, I wrote to Elvin asking whether I could spend some time in his laboratory.

After learning that I was also trained in engineering and applied mathematics and was interested in mathematical biophysics, Elvin suggested instead that we should meet once a week to analyze the known sequence data on antibodies in more detail, initially by writing them on long strips of paper and later by using computers. We reasoned that the variable region of light chains of immunoglobulins could have random amino acid substitutions just like other proteins. However, at the antibody-combining sites many more substitutions would be needed to accommodate the vast number of different antigens. Such amino acid variations were previously noted by Elvin as well as by Hilschmann and Craig, Putnam, Edelman, Franěk, Milstein and Pink, and others. We defined a quantitative measure, variability: Variability = Number of different amino acids at a given position/Frequency of the most common amino acid at that position.

When variability was plotted against amino acid position for the variable region of 70 human κ and λ and 7 mouse κ complete and partial light chains, the sequences of which were determined by many investigators, three distinct peaks bracketed by invariant or nearly invariant residues were seen at positions 24 to 34, 50 to 56, and 89 to 97, designated as hypervariable. There were insertions numbered 27A to 27F and others around position 95 now denoted by 95A to 95F based on more data.[3] We predicted that these, together with similar regions in heavy chains, would form the antibody-combining site. Now with many more sequences from various species of defined specificities and from inbred lines, the peaks in the variability plot have changed little.

The following year, we made a similar plot for the variable region of 37 complete and partial heavy chains, and again three distinct peaks were present as we had expected. Within a few years, our prediction was substantiated by three-dimensional structures of several hapten-binding Fab fragments, light chain dimers, and one Fv fragment, and the six hypervariable regions were named complementarity-determining regions (CDRs). More recently, a complex of lysozyme and an antilysozyme Fab fragment was crystallized, and its three-dimensional structure was determined to 2.8 Å resolution.[4] Fifteen amino acid residues from all six CDRs and two framework residues immediately adjacent to the CDRs made contact with the lysozyme molecule. Two other lysozyme-antilysozyme complexes recognized different epitopes and again all six CDRs were involved.[5,6] Currently, all X-ray structures of immunoglobulins have confirmed our prediction.

The fundamental contribution of our papers is the important prediction that antibody-combining sites are formed by the CDRs. It therefore becomes possible to design antibodies with desired binding properties using recombinant DNA techniques. Several investigators have replaced CDRs of human antibodies with those of mouse monoclonal antibodies with anticancer activities.[7] In addition, variability plots have also been used to try to locate CDRs in T-cell receptors,[8] to define residues that make contact with processed antigen and with T-cell receptor in the major histocompatibility complex class I molecules[9] and in the class I X-ray structure,[10] and to identify hypervariable regions of various strains of human immunodeficiency viral envelope glycoproteins.[11]

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