The Journal of Immunology

RESEARCH ARTICLE | NOVEMBER 01 2004

When Two-Dimensional Structure Led the Way

Norman R. Klinman

J Immunol (2004) 173 (9): 5333–5334.
https://doi.org/10.4049/jimmunol.173.9.5333

When Two-Dimensional Structure Led the Way

Norman R. Klinman[1]

In the current era, when proteins are sequenced by the thousands, it is difficult to appreciate the enormous impact the publication of a single sequence, that of the human myeloma protein Eu, had on the field of immunology in the 1960s. The *Proceedings of the National Academy of Sciences of the United States of America* article published in 1969 by Gerald Edelman and his coworkers (1) represented the culmination of a series of papers from that laboratory that delineated the two-dimensional structure of Eu and enabled an increasingly insightful interpretation of the structure/function relationships and genetic and evolutionary origins of the Ig molecule.

The accomplishments of the group led by Edelman were impressive even at the purely technical level. Although numerous laboratories at the time were involved in sequencing various proteins, including Ig, the work was tedious, requiring precise enzymatic and chemical cleavage, unblocking N termini, peptide sequencing by Edman degradation, and ultimately, alignment of the total sequence. Indeed, the H chain of Eu represented the largest fully sequenced polypeptide until that time. These technical accomplishments were facilitated by the insightful strategic approaches that enabled the collation of information obtained from enzymatic fragmentation, peptide analysis, chain separation, and ultimately, amino acid sequence (2–4). Progress in this endeavor was made possible primarily as the result of a series of seminal intellectual breakthroughs provided largely by the Edelman laboratory including the realizations that 1) obtaining accurate sequences would require homogeneous proteins, 2) myeloma proteins might provide the appropriate homogeneous counterpart to purified Abs, and 3) Bence Jones proteins, which could be obtained from the urine of myeloma patients, might represent an accessible and readily sequenced portion of the Ig molecule. Following the demonstration by Edelman and Gally (5) that Bence Jones proteins were homologous to Ig L chains, sequences of Bence Jones proteins provided the first insights into the existence of variable and constant L chain domains (6). In concert with this information, the information obtained from the complete sequence of the Eu H and L chains and the partial sequence of other myeloma proteins ultimately enabled the Edelman group to present a comprehensive view of the Ig molecule (see Fig. 1, Ref. 1) that has stood exceedingly well the test of time.

The elucidation of the two-dimensional structure of Ig during the decade of the 1960s helped resolve numerous longstanding issues in the field of immunology. 1) At the time the chain structure and sequence of myeloma proteins was emerging, the issue of the origins of Ab specificity was still highly controversial. The findings that the Ig molecule was comprised of two pairs of polypeptide chains and that the sequences of the amino terminal regions of these chains was extremely variable was a major factor in the determination that Ab specificity was the product of genetic diversity and clonal variability (clonal selection, Ref. 7), as opposed to Ag instruction of the folding of a relatively homogeneous set of proteins (instructional theories, reviewed in Ref. 8). 2) The finding that variable domains were sufficiently diverse to accommodate Ag discrimination supported the conclusion drawn from affinity labeling that these were the Ag-binding regions (9). 3) The alignment of the intrachain cystines within each domain suggested that each domain folded independently, thus providing the earliest insights into the three dimensional structure of Ig. 4) The presence of a cystine joining the H and L chain, so as to juxtapose their variable regions, lent credence to the concept that both chains contributed to Ag binding. 5) At the time of these studies, although variations in the functions of Ig isotypes such as their ability to fix complement were known, the basis for these differences was not known. The elucidation of the domain structure, which represented a major accomplishment of this manuscript, and the anticipation of the differences between the C_H domains of the various isotypes provided a structural rationale for these disparate functions. 6) The location of the cystines that tethered the two H chains in a nondomain homologous region between $C_H{}^1$ and $C_H{}^2$ ultimately led to the concept of a hinge region that allowed for flexible distancing of the two combining sites. 7) The delineation of the domain structure of Ig and the sequence correlations among the domains clearly implicated gene duplication in the evolutionary origins of Ig. 8) The structural juxtaposition of the variable and constant domains provided support for the theory that gene translocation was likely to play a role in Ig gene expression as suggested earlier by Dreyer and Bennett (10).

Identifying the lack of sequence homology between the V domains and the C domains, which all had significant sequence homology, suggested to Edelman et al. (1), that "each chain is specified by two genes, V and C," and enabled them to suggest the prescient conclusions that "translocation of genes may be the basis of the phenomenon of clonal expression," and that "irreversible differentiation and commitment of a lymphoid precursor cell may thus occur at the time of gene translocation."

In its totality, the body of knowledge provided by the studies that culminated in this *Proceedings of the National Academy of Sciences of the United States of America* article served as a major building block for future progress in almost all areas of immunology and earned Dr. G. Edelman the 1972 Nobel Prize for Physiology and Medicine, which he shared with Dr. R. R. Porter.

[1] Address correspondence and reprint requests to Dr. Norman R. Klinman, The Scripps Research Institute, 10550 North Torrey Pines Road, IMM-16, La Jolla, CA 92037. E-mail address: nklinman@scripps.edu

0022-1767/04/$02.00

References

1. Edelman, G. M., B. A. Cunningham, W. E. Gall, P. D. Gottlieb, U. Rutishauser, and M. J. Waxdal. 1969. The covalent structure of an entire γG immunoglobulin molecule. *Proc. Natl. Acad. Sci. USA 63:78*.
2. Edelman, G. M., and M. D. Poulik. 1961. Studies on structural units of the γ-globulins. *J. Exp. Med. 113:861.*
3. Fleischman, J. B., R. H. Pain, and R. R. Porter. 1962. Reduction of γ-globulins. *Arch. Biochem. Biophys. Suppl. 1:174.*
4. Nisonoff, A., F. C. Wissler, L. N. Lipman, and D. L. Woernley. 1960. Separation of univalent fragments from the bivalent rabbit antibody molecule by reduction of disulfide bonds. *Arch. Biochem. Biophys. 89:230.*
5. Edelman, G. M., and J. A. Gally. 1962. The nature of Bence-Jones proteins: chemical similarities to polypeptide chains of myeloma globulins and normal γ-globulins. *J. Exp. Med. 116:207.*
6. Hilschmann, N., and L. C. Craig. 1965. Amino acid sequence studies with Bence-Jones proteins. *Proc. Natl. Acad. Sci. USA 53:1403.*
7. Burnet, F. M. 1959. *The Clonal Selection Theory of Acquired Immunity*. Vanderbilt University Press, Nashville, p. 209.
8. Karush, F. 1962. The role of disulfide bonds in the acquisition of immunologic specificity. *J. Pediatr. 60:103.*
9. Wofsy, L., H. Metzger, and S. Singer. 1962. Affinity labeling: a general method for labeling the active sites of antibody and enzyme molecules. *J. Biochem. 1:1031.*
10. Dreyer, W. J., and J. C. Bennett. 1965. The molecular basis of antibody formation: a paradox. *Proc. Natl. Acad. Sci. USA 54:864.*