Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 1599–1600, March 1994

Commentary

An idiotope–anti-idiotope complex and the structural basis of molecular mimicking

Roberto J. Poljak

Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, 9600 Gudelsky Drive, Rockville, MD 20850

Idiotopes are antigenic determinants unique to an antibody or group of antibodies; they are defined serologically by the reaction of anti-idiotopic antibodies (Ab2) with the antibodies bearing the idiotopes (Ab1). The ensemble of idiotopes of an antibody constitute its idiotype. Idiotypes are useful markers to follow the appearance and persistence of specific antibodies and clones of cells in immune responses and the inheritance of immunoglobulin genes (reviewed in ref. 1). As a result of extensive studies on antibody primary structure, serology, and function, private (as opposed to public, or shared) idiotypes were shown to be associated, partially or entirely, with the complementarity-determining regions (CDRs) of antibody molecules. Idiotypes can span parts of CDRs combined with parts of "framework" or constant regions of antibodies. Thus, given the enormous potential variability of CDRs, idiotypes have an even larger potential for diversity which makes them an interesting model of self antigens.

The potential regulatory role of idiotype–anti-idiotype interactions in immune systems has been the object of many studies since the demonstration that a species (2) or an individual animal (3) is capable of producing antibodies that react with its own antibodies. Passive transfer of anti-idiotypic antibodies has been used to show that they can directly stimulate, enhance, or suppress the expression of idiotype-positive cells from the mature B-cell repertoire and inhibit the induction of idiotype-positive antibodies by specific antigens. Some of these observations were used in formulating a hypothesis for the regulation of the immune system through idiotype–anti-idiotype interactions (4). In addition, the existence of T lymphocytes with specificity for syngeneic idiotypes has suggested the possibility that antibody regulation could be effected by T cells reacting with specific idiotypes (reviewed in refs. 1 and 5).

Idiotypes associated with antibody heavy (H) or light (L) chains have been reported. However, their expression most frequently requires the association of the variable regions (V_H and V_L) of both chains. Since haptens can inhibit the binding of anti-idiotypic antibodies to combining-site-related idiotypes, it has been concluded that unique idiotypic specificities are associated with one or more of the CDRs of V_H and V_L. Sequencing and immunochemical studies have indicated that V_H residues in CDR2 and CDR3 are important in determining idiotypic specificities (reviewed in refs. 1 and 5). However, these studies could not provide the complete characterization of an idiotope in terms of the molecular structure of an antibody.

Since external antigens and anti-idiotypic antibodies can competitively bind to the same variable region of specific antibodies, some anti-idiotypic antibodies may carry an "internal image" of the external antigen. Functional idiotypic mimicry of ligands of biological receptors has indeed been described in several systems (reviewed in ref. 6). As an example, anti-idiotypic anti-receptor antibodies have been applied to identify putative receptors for the import of proteins into mitochondria. Only the use of additional techniques and criteria allowed these experiments to sort out the good from the bad candidates, prompting the question: mimics or gimmicks? (7). Further, the possibility of mimicking external antigens has led to proposals (8–10) to use anti-idiotypic antibodies as surrogate antigens. This possibility has attracted the attention of many laboratories, although no concrete results in terms of the production of widely used vaccines have been reported, but this is an extremely tough test of the internal-image hypothesis. A recent review (11) has discussed this topic from a structural viewpoint.

The three-dimensional structure of a private idiotope, and its relationship to the antigen-combining site of a monoclonal antibody (mAb), has been obtained at 2.5-Å resolution (12). This study required the determination of two crystal structures: (*i*) that of the Fab fragment from a mAb (Ab1) bound to its external antigen [lysozyme (13)] and (*ii*) that of same Fab Ab1 bound by the Fab from an anti-idiotopic mAb (Fab Ab2). The need to crystallize the same Fab Ab1 molecule in two different complexes illustrates one of the difficulties of this type of study. This work showed that the anti-idiotopic antibody and the external antigen completely overlap in their binding of the Ab1 (mAb D1.3); about half of the residues involved in antigen binding form part of the private idiotope, demonstrating a close correspondence between a private idiotope and the CDR.

An interesting structural study dealing with anti-idiotopes was published by Garcia *et al* (14). These authors utilized a system that had been extensively explored by Ronco and Verroust at the Tenon Hospital in Paris, in which a mAb (Ab1) against the octapeptide angiotensin II (Ang) was used to obtain polyclonal anti-antibodies (Ab2s), and these in turn were used to obtain an anti-anti-mAb (Ab3). Thus, Ab1 reacts with Ang, the Ab2s with Ab1, Ab3 with the Ab2s, and, as expected on the basis of the mimicry of external antigens by antibodies, Ab3 reacts with Ang. The crystal structure of the complex between Fab Ab3 and Ang was determined to 3-Å resolution. Ang does not have a unique structure in solution, but several lines of circumstantial evidence indicate that that bound by the Ab3 may well be the physiologically active Ang structure. In addition, the amino acid sequences of Ab1 and Ab3 were found to be very close, providing a structural basis for the fact that both mAbs bound Ang. This suggests that some structural feature of the polyclonal Ab2s, unavailable for study because of their molecular dispersity, should have mimicked the Ang structure. A search for conformations that approach that of the bound Ang indicated that the CDR3 peptide backbone of the human myeloma L chain Rei (15) does it with an rms distance of 0.8 Å. Thus, a CDR of the Ab2s could have elicited antibodies, one of which cross reacts with Ang and binds it with high affinity (7.4×10^9 M^{-1}). That CDRs may be involved in mimicking had already been reported (16) based on sequence homologies between the V_H CDR2 and V_L CDR2 of an anti-idiotopic antibody and the external antigen, the hemagglutinin from reovirus serotype 3/Dearing.

In this issue of the *Proceedings*, Ban *et al* (17), working in the laboratory of Alexander McPherson, report on the crys-

tal structure of a second idiotope–anti-idiotope Fab–Fab complex, at 2.9-Å resolution. The idiotope thus characterized occurs on a mAb, 730.1.4, specific for the E2 peplomer, a large glycoprotein of the feline infectious peritonitis virus, whose three-dimensional structure is not known. This idiotope of 730.1.4 includes amino acid residues from both its H and L chains, and mostly from the CDRs of the H chain. It extends over an area (calculated as excluded from solvent in the complex) of 860 $Å^2$, which is similar to that of the antigenic determinant of lysozyme in complex with D1.3 (810 $Å^2$) or of the idiotope of D1.3 described above (873 $Å^2$, values obtained if probed with a solvent molecule of radius 1.7 Å as by Ban *et al*). The interacting molecular surfaces are relatively flat. There are numerous van der Waals interactions and several hydrogen bonds. Thus, in its structural features this complex resembles a typical antigen–antibody complex.

The H chain of antibody 730.1.4 contributes 71% of the buried area of the idiotope, so that this idiotope is based predominantly on the H chain. Curiously, this antibody is reported to recognize an epitope on the E2 peplomer even when the protein is denatured in a Western blot, indicating that it is specific for an unfolded or "linear" sequence rather than a folded loop in the context of the tertiary structure of a protein. Be that as it may, the V_H and V_L CDR1s of the anti-idiotopic Ab2 show sequence homologies with the peplomer protein, suggesting that these may be the sequences and structures of the external antigen mimicked by the anti-idiotopic antibody. The V_L CDR1 sequence Val-Ser-Ser-Ser-Ile-Ser is homologous to the sequence Ile-Ser-Ser-Ser-Ile-Ser starting at position 276 of the antigen. The V_H CDR1 sequence Gly-Phe-Thr-Phe-Asn-Asn is homologous to the sequence Gly-Phe-Ser-Phe-Asn-Asn starting at position 1451 of the peplomer protein. Just as in the case of the anti-Ang antibodies discussed above, it is likely that loop regions of the antigen are implicated, loops that could more naturally be mimicked by the CDR loops of the anti-idiotopic antibody. The general structure of the epitope and how these loops are oriented in space would have to be established to verify the extent of mimicry and its molecular basis. Since such a demonstration has not yet been provided, we should remain open to this possibility but proceed with caution.

This report should be of interest to the numerous laboratories that have studied idiotypes and their possible role in immune phenomena.

1. Greene, M. I. & Nisonoff, A., eds. (1984) *The Biology of Idiotypes* (Plenum, New York).
2. Oudin, J. & Michel, M. (1963) *C.R. Acad. Sci. Paris* **257**, 805–808.
3. Rodkey, L. S. (1974) *J. Exp. Med.* **139**, 712–720.
4. Jerne, N. K. (1974) *Ann. Immunol. Inst. Pasteur* **125C**, 373–389.
5. Nisonoff, A. (1991) *J. Immunol.* **147**, 2429–2438.
6. Gaulton, G. N. & Greene, M. I. (1986) *Annu. Rev. Immunol.* **4**, 253–280.
7. Meyer, D. I. (1990) *Nature (London)* **347**, 424–425.
8. Nisonoff, A. & Lamoyi, E. (1981) *Clin. Immunol. Immunopathol.* **21**, 397–406.
9. Roitt, I. M., Cooke, A., Male, D. K., Hay, F. C., Guarnotta, G., Lydyard, P. M., DeCarvalho, L. P., Thanavala, Y. & Ivanyi, J. (1981) *Lancet* **i**, 1041–1045.
10. Sacks, D. L., Kelsoe, G. H. & Sachs, D. H. (1983) *Springer Semin. Immunopathol.* **6**, 79–97.
11. Mariuzza, R. A. & Poljak, R. J. (1993) *Curr. Opin. Immunol.* **5**, 50–55.
12. Bentley, G. A., Boulot, G., Riottot, M.-M. & Poljak, R. J. (1990) *Nature (London)* **348**, 254–257.
13. Amit, A. G., Mariuzza, R. A., Phillips, S. E. V. & Poljak, R. J. (1986) *Science* **233**, 747–753.
14. Garcia, K. C., Ronco, P. M., Verroust, P. J., Brünger, A. T. & Amzel, L. M. (1992) *Science* **257**, 502–507.
15. Epp, O., Colman, P., Felhammer, H., Bode, W., Schiffer, M., Huber, R. & Palm, W. (1974) *Eur. J. Biochem.* **45**, 513–524.
16. Williams, W. V., London, S. D., Weiner, D. B., Wadsworth, S., Berzofsky, J. A., Robey, F., Rubin, D. H. & Greene, M. I. (1989) *J. Immunol.* **142**, 4392–4400.
17. Ban, N., Escobar, C., Garcia, R., Hasel, K., Day, J., Greenwood, A. & McPherson, A. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 1604–1608.