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IMMUNOLOGICAL SPECIFICITY, INTERNAL IMAGES, AND THE ORIGINAL IDIOTYPIC SIN

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SUMMARY

The idiotype network hypothesis of Jerne led to the observation that some anti-idiotypic antibodies could mimic antigens reactive with the idiotype-expressing antibody. While such findings prompted some investigators to classify anti-idiotypic antibodies into relatively rigid classes, members of which either did or did not mimic antigen, consideration of relevant physical chemical principles suggests that mimicry should be more similar to a quantitative than a discrete variable. In addition, it is reasonable to expect that there are many ways to assess the extent of mimicry and these various measures of mimicry might not correlate perfectly. Immunochemical studies of antibodies specific for the cell wall polysaccharide of group A streptococcus and anti-idiotypes for these antibodies, as well as structural studies of diverse complexes between idiotypic and anti-idiotypic Fab or Fv fragments, are consistent with these conclusions.

INTRODUCTION

It is now more than four decades since *Fazekas de St. Groth* and *Webster* (1966a, b) reported their findings pertaining to the immunological phenomenon referred to as Original Antigenic Sin, the discovery of which they credited to *Francis* (1953). The basis of Original Antigenic Sin was the finding that vaccinees, previously infected by type A influenza virus, produced antibodies specific for the immunising influenza vaccine antigens but these antibodies reacted better with (or were of higher titre for) the antigens corresponding to the virus associated with the primary exposure through infection. Thus, Original Antigenic Sin refers to an immunological situation where the patterns of subsequent responses were significantly shaped by an initial immunological experience. In what follows, I suggest that the prior exposure of immunologists to certain concepts related to the phenomenon of idiotypy shaped much subsequent thinking about the nature of idiotypic recognition and mimicry of antigen by anti-idiotypes. It is appropriate to associate the word "sin" with these early notions because they were based on simplistic views of non-covalent interaction that have tended to hinder the adoption of more realistic conceptions of antigen recognition and anti-idiotypic mimicry.

THE IDIOTYPE NETWORK

In 1974, Niels Jerne published an extraordinarily influential review, in which he developed a then-novel view of the immune system as a network of clones functionally linked to each other through receptor-receptor (idiotype-anti-idiotype) interactions. Jerne's vision was exhilarating, transforming the immune system from a mere mechanism based on clone-versus-clone competition into a highly integrated, co-operative system with echoes of the central nervous system. Over the next two decades, many of the experimental directions stimulated by the network hypothesis proved equivocal if not futile. Ultimately, the immunological community grew tired of the unlimited layers of anti-idiotypic recognition that seemed pliable enough to rationalise any result, once obtained, but of limited value for prediction (*Greenspan*, 1997a). Nevertheless, there are concepts (e.g., molecular mimicry) and experimental results (e.g., identification of clonal populations of lymphocytes *in vivo*) pertaining to idiotype-anti-idiotype interactions that continue to be accepted and that relate to the rest of immunology and biology.

Central to Jerne's conception of the idiotypic network was the idea that in addition to antigen, any antibody variable (V) module (heavy and light chain variable domains), could bind or be bound by one or more other antibodies, termed anti-idiotypic antibodies (anti-Ids or Ab2). One can imagine that the pattern of thought that led Jerne to this notion bears a resemblance to the thinking that led others to the concepts of synthetic (*Geysen*, 1984; *Houghten*, 1985) or phage display peptide libraries (*Scott* and *Smith*, 1992). The central insight is that molecular recognition is a quantitative matter, and that it cannot reach perfection (*Naray-Szabo*, 1993). For example, intrinsic (monovalent) affinities of antibodies for antigens can range at least from 10^5 to 10^{11} L/M (*Karush*, 1978).

The followers of Jerne went on to classify anti-idiotypic antibodies into several categories based on the locations of the sites that they recognised on the idiotypic V module (reviewed in *Gaulton* and *Greene*, 1986). An anti-Id that bound to a site (idiotope) not overlapping with the antigen-binding site (or paratope) was to be called an α anti-Id or Ab2α. An anti-Id that bound to a site that overlapped with the antigen-binding site was to be called a β anti-Id or Ab2 It was assumed that the β-type anti-Ids would be able to mimic the antigen with respect to the idiotypic antibody. It should be noted that additional categories of anti-Ids have been proposed (*Gaulton* and *Greene*, 1986), but these categories did not find extensive use in the experimental literature.

INTERNAL IMAGES

The above ideas were appealing and easily grasped, and they stimulated an enormous wave of research designed to address how the idiotype network operated and how different kinds of anti-Ids contributed to this system. Of special interest were the β anti-Ids or Ab2β, also referred to as *internal images* by Jerne, which were believed to be important both for understanding the physiology of the idiotypic network and for clinical applications of anti-idiotypic antibodies, such as vaccine development (*Nisonoff* and *Lamoyi*, 1981; *Roitt* et al., 1981). It is perhaps paradoxical that the insights which led Jerne to explore

the implications of the previously described phenomenon of idiotypy were apparently abandoned in some of his conceptions relating to internal images and the mimicry of antigen by anti-Ids. Specifically, the conception of the internal image was developed as if guided almost solely by notions of molecular shape. The unfortunate consequences of this shape-centred thinking are chronicled in hundreds of articles in the immunology literature that appeared between the mid 1970s and the late 1980s.

What was largely missing in Jerne's conception of the internal image was the framework provided by the principles of physical chemistry, especially thermodynamics. This gap in Jerne's thinking may not seem surprising given that other immunologists were (are) in the habit of failing to incorporate such physical principles in their thinking. What makes it surprising, is that Jerne had done experimental work, early in his career, on the strength of antibody-antigen interaction, and had a long-term interest in physical chemistry (*Tauber*, 1994). It may well be that Jerne himself understood the limitations of his concept of the internal image, but many of those who were stimulated by his ideas appear to have been less aware of the caveats.

THE PHYSICAL BASIS OF BIOMOLECULAR RECOGNITION

The driving force for the formation of a non-covalent complex between biological macromolecules, or between a macromolecule and a small molecule, is the free energy change associated with the formation of the complex. What is often insufficiently appreciated is that this free energy change is derived from and applies to the entire chemical system in which the ligand or antigen (e.g., Id) and the receptor or antibody (e.g., anti-Id) are components. The solvent, counterions, and other solutes all can influence the free energy change for a particular interaction. Thus, the tendency of investigators to rationalise the intrinsic affinity between two molecules solely by analysis of the structural details of the intermolecular interface is to be discouraged despite its usefulness in some circumstances (*Greenspan*, 1992a). In the general case, conclusions relating to intermolecular affinity and derived only from information pertaining to the structural features of the complex will not be reliable.

Appreciation of another feature of the free energy change of complex formation is crucial to clear thinking about molecular recognition. The free energy change of complex formation reflects the relative stabilities of reactants and products. This point carries profound implications for any effort intended to determine the individual contributions of amino acids, atoms, or other sub-units of structure to a particular non-covalent interaction. The key point is that a mutation that has no direct effect on the energetics of the complex between antibody (receptor) and antigen (ligand), but that destabilises a reactant in the unbound state, will have the effect of increasing the affinity of the interaction. For some purposes, it may not be necessary to distinguish such mutations from those that directly stabilise the complex, while in other cases the distinction may well be important.

What, then, is an epitope, or antigenic determinant? The standard answer is that an epitope consists of the identities and spatial co-ordinates of the molecular sub-units (e.g., amino acids or atoms) that make physical contact with an antibody or T cell receptor. The problem with this definition is that in many cases, the immunologist is primarily interested in knowing which molecular sub-units contribute energeti-

cally to the cognate interaction or to the discrimination between cognate and non-cognate ligands (*Van Regenmortel*, 1989). These sets of sub-units are not necessarily identical with each other or with the set of contact residues (*Greenspan*, 1992a, 1997b). Thus, if the real interest is in these sets of energetic contributors, or in the residues for which substitution (through mutation) affects the affinity of binding or the differential affinities of binding for two or more ligands, then defining the contact residues is, in the general case, a poor substitute.

MIMICRY AS A QUANTITATIVE, MULTIDIMENSIONAL VARIABLE

How do these considerations pertaining to the nature of non-covalent interactions and epitopes affect an understanding of anti-idiotypic mimicry of antigen, or of molecular mimicry in general? An appropriate starting point is to consider what mimicry of an antigen (epitope) might mean. It is readily apparent that there are multiple reasonable senses of such mimicry (*Greenspan*, 1992b). Perhaps the most obvious form of mimicry of one molecule by another is on the level of purely structural resemblance. Two molecules are distinguishable by a chemist, and will have different names, as long as they are non-identical in composition or connectivity, even if the difference reduces to a single atom or covalent bond. It should not be surprising that any two such related molecules, referred to by different names, could share extensive elements of structure. However, even in the case of relatively unrelated molecules with respect to atomic composition, there is the formal possibility of regions of structure that are similar in three-dimensional distribution of electron density. Such similarity could arise from the identical atoms residing at more or less exactly the same relative positions or even from non-identical atoms occupying more or less equivalent relative positions in space over some patch of molecular surface. In this case, the mimicry would likely be limited to one region or face of each of the molecules being compared. Henceforth, the molecule being mimicked will be referred to as the model and the molecule exhibiting the mimicry will be referred to as the mimic.

A second conceivable form of mimicry might be termed immunochemical. This form of mimicry is concerned with non-covalent binding. The tendency to presume a strong correlation between structural and immunochemical mimicry should be resisted, not because such a correlation does not exist, but because the correlation is not reliable. Non-covalent interactions between biological macromolecules or between macromolecules and small ligands involve multiple factors, as noted above, and the most general approach presumes as little as possible. Once a given molecule, the mimic, is found to bind to any number of receptor molecules similarly to another molecule, the model, it may be of interest to know what structural relationship between mimic and model accounts for such similarity in binding. In some cases, it will be found that the structural similarities are not as impressive as some might have anticipated.

Consider the case of insulin, as reported by *Weiss* and colleagues (*Hua* et al., 1991). They note that there is a mutant form of insulin differing from the wild-type amino acid sequence by a single amino acid substitution. Furthermore, the crystallographic structure of

this mutant form of insulin is, with the exception of the one altered side chain, virtually identical to that of the wild-type form. Nevertheless, this molecule is not functional. In contrast, *Hua* et al. (1991) describe a point mutant of insulin that retains insulin function despite having a significantly different structure than the wild-type molecule by nuclear magnetic resonance (NMR) spectroscopy. Thus, this set of insulin molecules provides a compelling example of the potential for divergence between structural similarity and functional similarity.

The third form of mimicry that will be of strong interest to biomedical investigators is functional mimicry. In this instance, a biological response is elicited following non-covalent interaction between either the model or the mimic and, most typically, a macromolecular receptor of some sort. As in the case of immunochemical mimicry, this form of mimicry can be evaluated on its own operational basis, and it is best not to presume that a given degree of functional similarity necessarily reflects any given magnitude of structural or immunochemical mimicry.

It is not difficult to imagine molecules that elicit similar functional responses from different receptors on the same or different cells, or even different responses from the same receptor under different cellular or environmental circumstances. For example, binding to T cell receptors can have distinct consequences for immature T lineage cells in the thymus in comparison to mature T lymphocytes in the periphery. Another interesting example is provided by a study (*Tran Van Nhieu* and *Isberg*, 1993) of antibodies to a cell-surface integrin able to mediate uptake of attached particles, including the bacterial pathogen *Yersinia pseudotuberculosis*. The authors of this report found that different monoclonal antibodies specific for the integrin, as well as a protein expressed by *Y. pseudotuberculosis*, exhibited the ability to be endocytosed even though they bound to the receptor at distinct sites and therefore, presumably lacked a high degree of structural similarity. Presumably, in this case, the ability to bind to and cross-link the cell-surface integrin, regardless of the location of the contacted site on the integrin, is the basis for the similar functional activities of the molecules active in internalisation. Such degeneracy, in the relationships among different forms of molecular similarity, is also observed in cases where the endpoint is only binding, and not cellular function.

The non-functional insulin mutant, studied by *Hua* et al. and noted above, illustrates a general point worth noting: that, in general, the function of a molecule is not an intrinsic property of the molecule itself. Instead, the function of a molecule, X, is a property that depends on the relationships between X and the molecules with which X interacts. This important principle has also been demonstrated by studies of chimeric cell surface receptors that express the extracellular ligand-binding domain(s) of one molecule and the intracellular signal transducing domain(s) of another molecule. Consider two ligands (e.g., cytokines, growth factors, or hormones) X and Y, and their respective receptors, XR and YR. If the extracellular portion of XR is genetically fused to the intracellular portion of YR, and the chimeric receptor is expressed in a cell line, then it will not be surprising if the function of X with respect to the *X/YR*-expressing cell line (which does not express XR) is more like the function of Y than that of X.

With respect to structure, binding, and the elicitation of higher-order biological function, it is reasonable to determine not only whether there is or is not mimicry of a model molecule by a molecular mimic, but to what extent mimicry is exhibited. Any effort to

quantitate mimicry is likely to reveal that there are multiple ways of precisely defining the extent of mimicry. Consider functional mimicry. Binding to a cell surface receptor, such as an antigen-specific B or T lymphocyte receptor can have multiple consequences, such as proliferation or cytokine secretion. Each endpoint that can be envisioned can be used as the basis for the quantitation of the similarity in functional effects associated with either of the two molecules. There is reason to believe that these endpoints will not always correspond exactly. For example, *Evavold* and *Allen* (1991) demonstrated that changing a single amino acid in a peptide presented to a CD4+ T cell by class II major histocompatibility complex (MHC) molecule can result in activation of the T cell with respect to one function (IL-4 secretion) but not another (proliferation). In other words, the modified peptide is a better mimic of the original peptide for the elicitation of IL-4 secretion than for the elicitation of a proliferative response.

EXPERIMENTAL EVIDENCE PERTAINING TO ANTI-IDIOTYPIC MIMICRY

In this section, selected, but representative, experimental results pertaining to anti-idiotypic mimicry of antigen will be briefly discussed. First, highlights from my own studies on antibodies specific for the group-defining cell wall polysaccharide of group A streptococcus (*Streptococcus pyogenes*) will be summarised. This polysaccharide will be referred to as group A carbohydrate (GAC). Then, insights gained from some of the first crystallographic structures of Id-anti-Id complexes are discussed.

From a large panel of monoclonal antibodies (mAbs; more than 30) secreted by hybridomas and specific for GAC, one mAb (HGAC 39) was chosen to immunise rats for the production of anti-idiotypic antibodies. Nine monoclonal anti-idiotopes specific for the variable module of HGAC 39 were produced and characterised (*Greenspan* and *Davie*, 1985a). Each monoclonal anti-Id exhibited a unique reactivity pattern with the HGAC mAbs. One, anti-IdX, reacted almost equivalently with about half of the panel of GAC-specific mAbs and failed to react detectable with the remaining GAC-specific mAbs. A variety of studies, using different methods, suggested that anti-IdX bound far from the paratope of HGAC 39, and this conclusion was directly confirmed by electron microscopy of negatively stained Id–anti-Id complexes (*Roux* et al., 1987). Such an anti-Id would not be expected to be an impressive mimic of the cell wall polysaccharide epitope. In contrast, another monoclonal anti-Id, anti-IdI-3a, bound to a large fraction of the HGAC mAb panel with varying effectiveness (*Greenspan* and *Davie*, 1985b). The assays that suggested the proximal location of the idiotope recognised by anti-IdX also suggested the distal location of the idiotope recognised by anti-IdI-3a. Electron microscopic analyses supported this conclusion. Since the interaction between HGAC 39 and anti-IdI-3a was directly inhibited by free N-acetyl-glucosamine (GlcNAc), a dominant component of the GAC epitope recognised by HGAC 39, it seemed reasonable to entertain the notion that anti-IdI-3a was a Ab2β, or internal image.

Several results suggest why a dichotomy, that classifies anti-idiotopes as internal images or not, is simplistic. First, while available results suggest

that GAC and anti-IdI-3a mutually compete for binding to overlapping regions of the HGAC 39 variable module, anti-IdI-3a binds to varying degrees to GAC-specific mAbs that all react comparably with GAC. Furthermore, anti-IdI-3a probably binds with considerably higher intrinsic affinity to HGAC 39 than do GlcNAc or GAC. When C57BL/6J mice were immunised with three isotype-matched anti-Ids specific for HGAC 39, anti-IdI-3a elicited the more GAC-specific serum antibodies than the other two anti-Ids or control rat IgG (*Monafo* et al., 1987). Thus, using induction of a primary antibody response to GAC in C57BL/6J mice as the measure of mimicry, anti-IdI-3a was the best mimic among the anti-Ids tested. Interestingly, when primary immunisation of C57BL/6J mice with anti-Id was followed by immunisation with whole heat-killed, pepsin-treated group A streptococci, the anti-Id that was most effective at priming for a secondary response to GAC was anti-IdX. Therefore, under the conditions of this particular immunisation protocol, and with respect to this measure of mimicry, anti-IdX was a better mimic of GAC than anti-IdI-3a, despite the fact that anti-IdX binds to the HGAC 39 at a site distinct, and relatively distant from, the antigen-binding site.

This brief summary of the studies on the antibodies and anti-Ids in the GAC system suggests that mimicry has many measures or dimensions (*Greenspan* and *Bona*, 1993). One of a set of anti-Ids may be the best mimic with respect to one such measure and yet not be the best with respect to another. Furthermore, there is no certain relationship between the extent to which idiotope and paratope overlap and the extent of immunochemical or functional mimicry of antigen by anti-Id *Greenspan* and *Roux*, 1987).

The crystal structures of several complexes between idiotypic and anti-idiotypic Fab or Fv fragments have been studied (e.g., *Bentley* et al., 1990; *Ban* et al., 1994; *Evans* et al., 1994; *Fields* et al., 1995). For the purpose of understanding anti-idiotypic mimicry of antigen, the study of *Fields* and colleagues (1995) is the most relevant, as the anti-Id in this study, E5.2 is able to mimic the antigen, hen egg lysozyme (HEL), in eliciting HEL-reactive antibodies when injected into mice.

The crystal structure of Fv fragments of E5.2 and the HEL-specific mAb, D1.3, reveals many contacts that are similar to those in the D1.3-HEL complex. While the extent of such similarities is impressive, it is equally important to note that the two complexes have numerous differences. For instance, while six hydrogen bonds between the Fv fragments of E5.2 and D1.3 are superimposable on hydrogen bonds of the D1.3-HEL complex, seven are not so superimposable. While 13 residues of D1.3 that contact E5.2 also contact HEL, there are five D1.3 residues that contact E5.2 that do not contact HEL and four D1.3 residues that contact HEL that do not contact E5.2. Thus, even in this most impressive example of structural and functional mimicry, the structural mimicry cannot be considered complete.

CONCLUSION

The notion that anti-Ids can be readily classified into two (or even a few) categories that will correlate with the locations of the corresponding idiotopes on the idiotypic V module is too simple to handle the reality of Id-anti-Id interactions. Such interactions, after all, like other non-covalent interactions, can

vary over a spectrum of affinities and arise from different combinations of atomic level forces. Furthermore, thermodynamic considerations suggest that the affinity and specificity exhibited by a given Id-anti-Id pair are the result of factors that are not obvious solely from detailed analysis of the interface created by formation of the Id-anti-Id complex. Thus, one of a group of anti-Ids may mimic an antigen better than the other anti-Ids with respect to one measure of mimicry and less well with respect to a different measure of mimicry. When the dimensional and quantitative complexities of such interactions are acknowledged, the loss in simplicity is balanced by an increased ability to describe the experimental realities.

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