Natural Id-Anti-Id Networks and the Immunological Homunculus

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It is postulated by Jerne and his associates that a network of mutually interacting idiotypes (ids) and anti-idiotypes (anti-ids) is a major factor in regulating the immune response [1]. Analogous to the nervous system wherein an environmental stimulus becomes information as a result of setting into motion self-connected neural networks, the environmental stimulus of the immune system, the antigen, acquires meaning by impinging on self-recognizing id-anti-id networks. Although the id-anti-id hypothesis does not explain or necessitate other demonstrably important immune system factors such as MHC molecules or helper T cells, the notion has fruitfully roused the neural networks of many immunologists.

The aim of this article is to state the lessons my colleagues and I have learned by studying id-anti-id networks of two sorts: one sort expressed by interacting antibodies, the other by interacting T cells. My aim is to draw attention to observations that might be considered by those proposing network solutions to immune regulation problems.

1. Id as Internal Signal or Id as Antigen

A fundamental distinction can be drawn between an id as a network connector or signal internal to the immune system, and an id as an antigen. An id may be perceived by the immune system as any other immunogenic macromolecular structure capable of stimulating the production of antibodies. To the extent that these antibodies are specific for the id, they may be termed anti-ids. In practice, the isolated id is often purified and injected in an aggregated form together with a strong adjuvant [2]. The resulting anti-id in turn is then isolated , purified and used with a suitable adjuvant to immunize yet a third set of animals, that in turn respond by making an anti-anti-id. There seems to be no end to the chain of antibodies that may be generated by such contrived immunization and this led Jerne to postulate in his original formulation that the idiotypic network was open-ended and proceeded until it fed back upon itself [1].

An id as network connector is quite another creature. Here one should be dealing with ids, anti-ids, anti-anti-ids, etc. arising in a single individual and accompanying or perhaps even preceding the response to a designated antigen. We have focused our studies on natural id networks. Natural networks, as we discovered, may differ considerably from contrived networks.

2. An Antibody Network: The Response to Insulin

My colleagues and I have investigated two types of natural id-anti-id networks related to insulin; that evoked spontaneously by immunizing mice or guinea pigs to ungulate insulins [3-7], and that appearing in mice, rats or humans spontaneously developing autoimmune diabetes. The details of these systems have been or are about to be published and I shall not describe the experiments here, only their meaning. Suffice it to say that the epitope that triggers the network is the portion of the insulin molecule bound by the insulin hormone receptor. This epitope is highly conserved, if not

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identical in most mammalian species, with the notable exception of the guinea pig and her close relatives (the histricomorphs) who have a markedly aberrant insulin molecule [8].

The idiotype, which we have designated the DM-id in recognition of D. Elias and M. Rapaport who isolated the first DM positive monoclonal hybridomas, binds the conserved epitope and thus mimics the insulin hormone receptor [9] The specific anti-idiotype, the anti-DM-id, which arises spontaneously after the appearance of the DM-id, mimics the insulin epitope and binds to the insulin hormone receptor, and thus activates the biochemical effects of insulin itself. In vivo, the anti-DM-id causes hypoglycemia when it first appears, but after about a week it produces down-regulation and desensitization of insulin receptors. This causes peripheral resistance to insulin [10]. Thus, the DM network is significant pathologically as well as interesting immunologically. What has it taught us about immune regulation?

3. The Natural Network Is Closed

In contrast to the openness of contrived networks, the natural DM network seems to be closed; it does not appear to extend beyond the anti-id. It may be claimed that the anti-anti-id and the anti-anti-anti-id etc. occur but we miss them for lack of sufficiently sensitive probes. I can only answer that we looked for them in vain.

The natural DM network is closed in another sense; as far as we can tell it is inducible only once in the adulthood of otherwise healthy mice. The DM-id is produced only transiently, on days 6-13 of the primary response to immunization with insulin [4]. We never succeeded in detecting the DM-id subsequently despite hyperimmunization of the mice [11].

Similarly, the anti-DM-id was observed to appear only once, on days 24-40 after immunization [4]. It could not be induced a second time by repeated immunization of healthy mice to insulin [11].

Animals spontaneously developing diabetes such as NOD mice or BB rats spontaneously develop both the DM-id and the anti-DM-id [11]. Unlike healthy mice, these creatures persist in producing both DM-id and anti-DM-id. Thus, the development of autoimmune diabetes is associated with a persisting (dyregulated?) network.

4. The Immune Response is Partial to the DM-id

In contrast to contrived anti-ids, which may be induced by artificial immunization to apparently any id, upon immunization to insulin a spontaneous anti-id was detected only to the DM-id. Although insulin-immunized mice make a variety of different antibodies to insulin negative for the DM-id, no spontaneous anti-ids were observed for these DM-negative antibodies. The bias for the DM-id can not be explained by the fact that the DM-id is an autoantibody; most of the antibodies that mice make to ungulate insulins can be absorbed by mouse insulin. Thus, even the DM-negative anti-insulin antibodies include autoantibodies; nevertheless, DM-negative ids do not appear to be regulated by anti-ids.

5. The Natural DM Network is Conserved

The partiality of the immune system towards the DM-id is also evident in the cross-species conservation of this network. We detected the DM-id in mice of all strains

responding to insulin, in rats [11], in guinea pigs [6,7] and in humans [12]. Indeed, mouse monoclonal DM-ids can be used as reagents for detecting human anti-DM-ids and mouse anti-DM-ids can be used to detect human DM-ids [9]. Thus the DM-id network is both dominant within a species and conserved in evolution.

6. The Immunological Homunculus

Why is this insulin antibody, the one we call the DM-id different from all other insulin antibodies? Implicit in this question is the realization that not all ids are created equal, at least in the eyes of the network. Since DM-positive and DM-negative ids may function equally as autoantibodies, that is they bind to self-insulin, we may also conclude that not all autoantibodies are dealt with by the same regulatory mechanisms. In the eyes of the immune system, why does one autoantibody enjoy special privileges?

In pondering this question it is worth noting the fact that guinea pigs upon immunization to ungulate insulins produce the DM-id; but unlike mice they don't turn off the DM-id or make an anti-DM-id [6,7]. Hyperimmunized guinea pig anti-insulin antiserum is rich in DM-id; anti-DM-id is undetectable. Recall that guinea pigs express an insulin molecule that has mutated away from the standard shape; it is studded with mutations in the conserved portion leading to a loss of about 99% of its ability to interact with the standard insulin hormone receptors characteristic of other mammals [8]. In short, guinea pig insulin lacks the DM epitope. As a consequence, guinea pig insulin cannot trigger the DM network in mice. Ungulate insulin does bear the DM epitope and so triggers the DM-id both in mice and in guinea pigs. But only mice go on to make the anti-DM-id. This suggests that it is not the mere presence of the DM-id that tells the network to make an anti-DM-id; rather the structure of the individual's own insulin (mouse versus guinea pig) bears the information. Thus, the network may be fashioned not around the immunizing epitope, but rather around the structure of the self; or to be more precise, around certain favored structures of the self.

To generalize beyond the response to insulin, natural anti-id networks seem to be more readily inducible by epitopes that are ligands for certain physiological receptors. For example, immunization to ligands of the acetylcholine receptor induces anti-ids that recognize the acetylcholine receptor [13]; immunization to thyroid stimulating hormone (TSH) induces anti-ids that recognize the TSH receptor [14]. In short, the natural network seems to favour structures composed of the active sites of physiological ligands so that the anti-id may behave as a ligand for a receptor (non-immune).

It is doubtful that there is a selective advantage in making an anti-id that acts like an anti-receptor antibody; on the contrary there is probably a physiological disadvantage. Therefore, it is conceivable that the network makes such an anti-id the better to control it, to guarantee that if it does get made, then it gets made only once. Recall that this is the case in normal mice. In contrast, diabetes prone mice and rats, and possibly humans, have a problem in regulating the anti-DM-id.

Be that as it may, the natural anti-DM-id is surely an internal immunological image of the functional portion of the individual's own insulin molecule. Other, non-DM domains of the individual's insulin are not represented in the network. Thus the network creates a highly selective representation of certain body structures. This recalls the homunculus, the "little man" engraved in the motor and sensory areas of the brain cortex. The picture of the little man is topologically distorted; it does not represent the space or volume occupied by the particular organ, but instead gives

weight to the functional importance of the organ. (The homunculus in the human brain has giant thumbs and vocal cords, the dog's has a big nose). The brain uses its internal little man to sort and process nervous information.

The immune system also could, probably must have its little man to consult in processing information, particularly to aid in deciding what is self. As the insulins of humans and mice have a common DM epitope, they both share a common DM shape in the immunological homunculus. The guinea pig's immunological homunculus has a different picture of insulin because the guinea pig has a different self-insulin.

In addition to insulin, humans and mice share other macromolecular similarities, and this could explain idiotypic similarities between human and murine autoantibodies in systemic lupus erythematosis (SLE) or other autoimmune conditions. A new mouse model of SLE discovered by S. Mendlovic and his colleagues illustrates the importance of network interactions in the induction of autoimmunity [15]. Mice of a strain that does not spontaneously develop SLE were found to develop the disease along with its characteristic complement of various autoantibodies following immunization with a human monoclonal antibody bearing a common idiotype associated with human SLE. In other words, the anti-idiotypic response of the mice to the human-SLE idiotype unleashed SLE with all its immunologic manifestations. Is there a pre-formed SLE-network lying dormant in the immunological homunculus?

Obviously within each species there is even greater uniformity of functional macromolecules, and thus of the immunological homunculus. The common nature of the immunological homunculus may explain why humans with a common autoimmune disease produce autoantibodies to the same epitopes and, perhaps why these epitopes are often functionally important enzymes. For example, thyroid peroxidase is a major autoantigen in thyroiditis [16], lipoate acetyltransferase in primary biliary cirrhosis [17], and a cytochrome enzyme in chronic active hepatitis [18]. Anti-nuclear antibodies also may be directed against enzymes [19]. Is it an accident that the target antigens of autoimmune reactions are not only shared but functional?

7. The T Cell Network

To serve as a reference for interpretating and evaluating incoming antigenic signals, the immunological homunculus, like the neurological homunculus, must be formed before the antigenic signals enter the system. To put it another way; an epitope demonstrates its immunological dominance when it is preferred above alternative epitopes as the target for an immune response. The DM epitope is dominant, at least initially, because the immune system is receptive; the immunological homunculus anticipates the DM epitope.

How then is the immunological homunculus encoded? How can the DM-id know that it should be made even before immunization to insulin? To find an answer to this question we have begun to measure the responsiveness of T cells to monoclonal DM-id and anti-DM-id antibodies. More experiments are needed to draw firm conclusions, nevertheless the results thus far have raised the possibility that the immunological homunculus may be encoded in the reactivities of T cells.

The experiments involved culturing spleen or lymph node cells from mice with monoclonal DM-id or anti-DM-id antibodies and measuring the incorporation of labelled thymidine into DNA as a measure of T cell reactivity during various stages in the response to insulin. The results showed that naive mice had slight but significant T cell reactivity to the DM-id before being immunized to insulin. There was no reactivity

to the anti-DM-id or to a DM-negative anti-insulin antibody. A week after immunization to insulin, at the time of the appearance of the DM-id antibody, there was an increase in T cell reactivity to the DM-id. This reactivity waned and disappeared as there arose T cell reactivity specific for the monoclonal anti-DM-id. This anti-(anti-DM-id) reactivity peaked at the time of the peak in anti-DM-id antibody and then it too declined; but it did not disappear. For at least 6 months after immunization to insulin, anti-(anti-DM-id) T cell reactivity was detectable. Anti-(DM-id) T cell reactivity was no longer observed during this time. Thus, the DM-id antibody was preceded by anti-(DM-id) T cell reactivity and the anti-DM-id antibody was accompanied by anti-(anti-DM-id) T cell reactivity. Persistence of the anti-(anti-DM-id) T cell reactivity was associated with resistance to reinduction of the DM network. In other words, the dominance of the DM network was associated with preexisting anti-(DM-id) T cells and permanent down-regulation of the DM network with persistence of anti-(anti-DM-id) T cells.

T cells, among their other functions, are regulators of antibody production by B cells. Therefore, it is reasonable to suppose that the behavior of the DM antibody network, perhaps even its existence, is founded on T cell activity. In a fundamental sense, the DM antibody network might be encoded in a T cell network.

My associates and I are presently studying a second example of immunological dominance associated with preexisting anti-idiotypic T cell reactivity, that related to the 65KD heat shock protein (hsp65). The hsp65 molecule is a major immunodominant antigen in Mycobacteria [20]. Persons immunized to M. tuberculosis or M. Leprae make immune responses primarily to epitopes on hsp65. This is unexpected because hsp65 is a highly conserved molecule and there is a very close sequence homology between mammalian and bacterial hsp65 molecules. Parts of bacterial hsp65 probably look like self-epitopes to the immune systems of mammals. Indeed, immunity to bacterial hsp65 is associated with autoimmune arthritis both in rats (adjuvant arthritis; [21]) and in humans (rheumatoid arthritis; [22]).

Mycobacteria express about 10^4 genes furnishing the mammalian immune system with a very large number of safely foreign epitopes. Why should the immune response focus with such consistency and vigor on hsp65, which looks like self to the extent that it may arouse autoimmunity? On the contrary, the principle of horror autotoxicus should lead one to expect hsp65 to be a very poor immunogen. (The fact that this expectation is contradicted by reality should by itself suggest that our basic ideas about self-tolerance may be in need of revision.)

To investigate the T cell immune response to hsp65 we developed a T cell line, designated M1, specific for the hsp65 molecule. M1 seems to exemplify a major shared anti-hsp65 T cell idiotype. Relevant to the present discussion is the observation that naive rats express a slight but significant degree of T cell reactivity, not to hsp65 itself, but to the anti-hsp65 M1 line. In other words anti-(anti-hsp65) T cell reactivity preexists, anticipates as it were, immunization to the mycobacterium and its hsp65 antigen. After immunization to the whole mycobacterium the response to the anti-hsp65 idiotype actually flares up sooner (by day 4) than does the response to the hsp65 molecule or other mycobacterial antigens. Later however (by day 10) the magnitude of the T cell response to the hsp65 antigen surpasses that of the T cell response to the M1 anti-hsp65 idiotype.

Thus, natural T cell anti-idiotypic reactivity precedes the immune response to the hsp65 antigen as it does the triggering of the DM antibody network. Hence, the observation of preexisting T cell anti-idiotypes is not unique to the response to the DM epitope of insulin and may, upon further investigation turn out to be a general

phenomenon. Perhaps the immunological homunculus is encoded in such unsolicited T cell reactivity. H. Atlan and I have recently developed an automaton model of a T cell regulatory network integrating anti-idiotypic T cells, which recognize the effector T cell, with helper and suppressor T cells, which recognize the antigen [23]. A number of such regulatory units organized around key self-antigens could comprise the homunculus.

8. Problems and Paradoxes

The immunological homunculus described here is composed of a limited set of spontaneously reactive anti-idiotypic T cells that function to enhance the immunological dominance of certain self-epitopes or self-mimicking antigens. The result of this dominance is tight regulation, although dysregulation and autoimmune disease involving the dominant antigen occurs in some relatively few individuals. This formulation leaves us with a number of questions for experimental and theoretical consideration.

Deletion of autoreactive T cells is shown to take place, probably in the thymus [24]. How then can self-tolerance be regulated in practice by the apparently mutually exclusive mechanisms of deletion on the one hand and spontaneous heightened autoreactivity on the other hand? Are there fixed classes of self-antigens handled in one or the other way? If so, what decides which self-antigens are regulated by clonal deletion and which by anti-id networks?

It is not only the self-antigens that are problematic. T cells seem to recognize epitopes composed of relatively short peptide segments of the conventional antigen fixed in a cleft of an MHC molecule [25]. Does a regulatory anti-idiotypic T cell also recognize an MHC molecule with a small processed peptide segment of the T cell receptor or antibody idiotype, or does the T cell recognize the structure of the idiotype itself? If the latter were true, then the binding site of the anti-idiotypic T cell receptor would mimic the structure of antigenic epitope. Such a preexisting T cell could be understood to prime the response to the specific epitope. It would be more difficult to envision how a peptide-recognizing T cell could prime the immune system for a response to the antigen itself. As yet the anti-id T cells have not been cloned and we have no definitive answer to the id recognition question: peptide-MHC or unprocessed id. Nevertheless, the DM-id network does not appear to be MHC restricted - mice of all H-2 genotypes responding to insulin make the same DM network; a finding which does not support, but also does not contradict the peptide-MHC view of T cell recognition.

9. T Cell Vaccination

Although much remains to be clarified regarding the functioning of anti-idiotypic T cells in regulating the immune system, it has been possible to mobilize such T cells to prevent or treat autoimmune disease. Autoimmune effector T cells responsible for mediating particular autoimmune diseases in experimental animals when suitably treated can be used as vaccines to elicit or augment the activity of specific anti-idiotypic T cells; a procedure termed T cell vaccination [26] . The vaccinated animals develop heightened anti-idiotypic T cell responses to the pathogenic T cell clones responsible for the disease and thereby the disease is prevented or suppressed [27]. The effectiveness of T cell vaccination in animal models has provided the rational for its application to human autoimmune disease [28]. One might reason that the usefulness of T cell vaccination for medicinal purposes rests on the natural physiology of T cell anti-idiotypy in immunological control.

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