Chapter 9. First symmetry

He hasn't an enemy in the world,
and none of his friends like him.
-Oscar Wilde with reference to Bernard Shaw,
Sixteen self sketches (1949)

The view of the immune system as a set of diverse, independent clones, each clone ignoring and being ignored by all the others, lasted from the birth of clonal selection in 1957 (Talmadge, Burnet), to the birth of network theory in 1973 (Jerne). Experimental evidence supporting the idea that the immune system is a network of functionally connected clones accumulated rapidly during the early 1970's. The Richter theory took the field a big step further, by demonstrating at a theoretical level that the connections can have functional consequences. Richter's theory adopted from Jerne the assumed asymmetry in V-V interactions. The next step was to question that assumption, and the discovery that without it a more powerful theory can be constructed.

The asymmetry of the Jerne picture

There is an asymmetrical interaction between an antigen and the clone of lymphocytes that recognizes it (the Ag-V interaction). The antigen stimulates proliferation of a clone, and the antibodies produced by the clone exert a negative influence (eliminate) the antigen. When Jerne first drew a picture of the network (Figure 8-1) he incorrectly extrapolated this Ag-V asymmetry to the V-V interactions between idiotypes (acting as an antigen) and antiidiotypes (the clones that recognize the idiotypes). He emphasized the supposed asymmetry by symbolizing each antibody V as a dumbbell, with two functionally distinct parts, namely the paratope (recognizing part) and an idiotope (recognized part). A paratope of one antibody was thought to recognize an idiotope on another antibody, while the idiotope did not recognize the paratope. Hence idiotypes would stimulate antiidiotypes, but antiidiotypes would not stimulate idiotypes. In 1982 he retreated from the strict asymmetric aspect of his model somewhat and claimed "novelty [in the] proposal that denies the existence, on a variable domain of a specially constructed combining site or combining region. We replace this customary assumption by the notion that any area of a variable domain, which happens to be sufficiently complementary to an epitope or idiotope displayed by another molecule, may serve as combining site, or paratope" (reference 82).

The original mistaken asymmetry was based partly on information that was available at the time about the shape of the V region. It was known that when haptens bind to V regions they typically fit into a cleft, so it was tempting to assume that the same sort of geometry would apply to V-V interactions. The

paratope was accordingly assumed to be essentially concave. This also made the paratope analogous to the active site of an enzyme, which was typically a cleft. (This is known as the lock and key analogy for enzymes and their substrates). The idiotope was therefore assumed to be convex, so that a V-V interaction would constitute a concave-convex fit, and we would have the geometric basis for an asymmetric relationship.

Symmetry in V-V interactions

In the case of V-V interactions, however, both a top-down approach and a bottom-up approach showed that the interactions are typically symmetric. The bottom-up approach includes data on symmetric stimulation and on symmetric killing that we will now review, together with structural X-ray data of V-V interactions.[90,91] The X-ray data shows two irregular three-dimensional shapes with enough complementarity to give binding, and there is no way to say which is the "lock" and which is the "key", or which is the “idiotype” and which is the “antiidiotype”.

The necessity for cross-linking of receptors in B cell stimulation is shown for example by the experiments shown in Figure 9-1. Antibodies to B cell receptors are able to cross-link receptors and stimulate the cells to proliferate, and likewise divalent $F(ab)_2$ fragments of these antibodies are stimulatory.[92,93,94] On the other hand, univalent Fab fragments are unable to cross-link the receptors and cause no proliferation. In addition, a combination of the Fab fragments that bind to the receptors and divalent anti-Fab antibodies can cross-link receptors and do cause proliferation. Finally, Fab molecules with the anti-Ig specificity inhibit the stimulation by anti-Ig.

Further evidence for the cross-linking of receptors playing a role in the stimulation of B cells lies in the fact that anti-constant region (anti-Ig) antibodies, anti-allotype antibodies, antiidiotype antibodies and antigen can all

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[90] G. A. Bentley, G. Boulot, M. M. Tiottot et al., (1990) "Three-Dimensional Structure of an Idiotope-Anti-Idiotope Complex," Nature, 348, 254-257.

[91] N. Ban, C. Escobar, R. Garcia, K. Hasel, J. Day, A. Greenwood and A. McPherson (1994) "Crystal Structure of an Idiotype-Anti-Idiotype Fab Complex," Proc Natl Acad Sci USA, 91, 1604-1608.

[92] M. W. Fanger, D. A. Hart, J. V. Wells, and A. Nisonoff (1970) Requirement for cross-linkage in the stimulation of transformation of rabbit peripheral lymphocytes by antiglobulin reagents. J. Immunol., 105, 1484-1492.

[93] C. Koch and H. E. Nielson (1973) Effect of anti-light-chain antibodies on rat leukocytes in vitro. Scand. J. Immunol. 2, 1-8.

[94] V. C. Maino, M. J. Hayman, and M. J. Crumpton, (1975) Relationship between enhanced turnover of phosphatidylinositol and lymphocyte activation by mitogens. Biochem. J. 146, 247-252.

be stimulatory. These findings are inconsistent with the wide-spread notion that there is a site on the antibody-like receptor of the B cell (the "antigen-binding site") that is unique, in that it alone can interact with an antigen. Rather, anything that is at least divalent and is able to bind to an exposed part of the receptor has the potential to cause cross-linking and stimulate the cell.

The cross-linking postulate also means that if an idiotype is stimulatory for a cell expressing the corresponding antiidiotype, the antiidiotype can be expected to be stimulatory for a cell expressing the idiotype. This symmetry is a fact of paramount importance in the development of network theory.

In 1975 two experimental papers were published that supported the existence of symmetrical stimulatory interactions. Eichmann and Rajewsky[95] reported that they could prime both B cells and T cells bearing a particular idiotype using antiidiotypic antibodies. Trenkner and Riblet[96] found that they could induce antibody production in vitro using antiidiotypic antibodies. This was direct evidence of stimulation in the direction that was unexpected in the context of the Jerne picture. Meanwhile Köhler[97] proposed that the idiotypic interactions in the immune system are symmetrical, and my first symmetrical network theory paper was published.[98]

Anti-anti-X can resemble X

Symmetrical V-V interactions are also supported by the following thought experiment. Consider all the clones that recognize a particular protein antigen X. Anti-X clones stimulated by the antigen have an average shape that is complementary to the shape of the antigen. These clones would stimulate a set of anti-anti-X clones that in turn have an average shape that is complementary to anti-X and therefore resembles X. If anti-anti-X resembles X, it is not surprising that anti-anti-X should be able to stimulate anti-X clones, as seen experimentally by Eichmann and Rajewsky and by Trenkner and Riblet.

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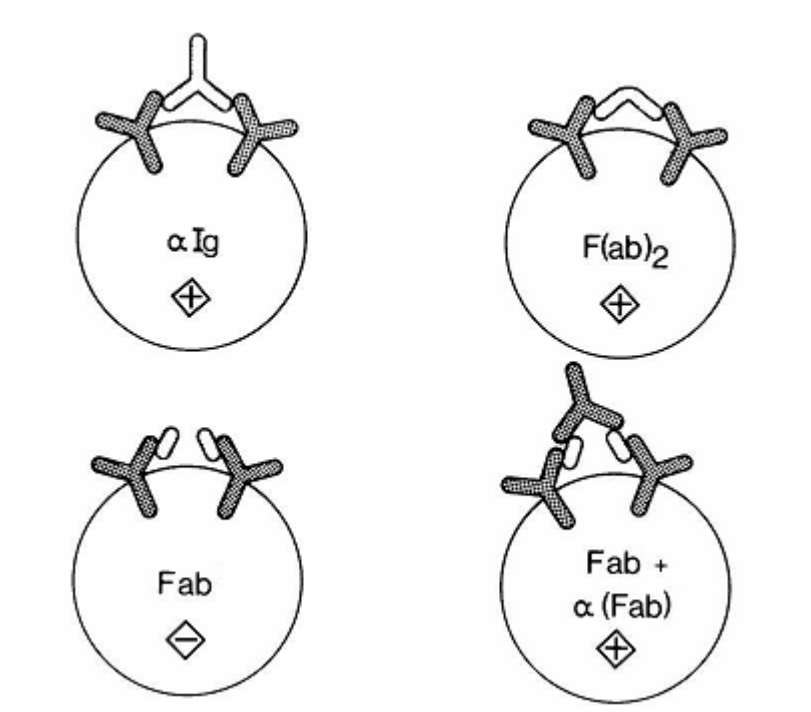
[95] K. Eichmann and K. Rajewsky (1975) Induction of T and B cell immunity by antiidiotypic antibody. Eur. J. Immunol. 5, 661-666.

[96] E. Trenkner and R. Riblet (1975) Induction of antiphosphorylcholine antibody formation by antiidiotypic antibodies. J. Exp. Med. 142, 1121-1132.

[97] H. Köhler (1975) The response to phosphorylcholine: dissecting an immune response. Transplantation Reviews, 27, 24-56.

[98] G. W. Hoffmann (1975) “A network theory of the immune system.” Eur. J. Immunol., 5, 638-647, 1975

Figure 9-1. The fact that cross-linking of receptors stimulates B cells to proliferate is demonstrated in experiments using anti-immunoglobulin (α-Ig) (divalent, cross-links receptors, causes proliferation), Fab fragments of α-Ig (monovalent, cannot cross-link receptors, do not cause proliferation), $F(ab)_2$ fragments of α-Ig (divalent, cross-links receptors, causes proliferation) and Fab fragments of α-Ig together with anti-Fab antibodies (causes proliferation). Reproduced from G. W. Hoffmann (1980) Contemp. Topics in Immunobiol. 11, 185-226.



Mimicry of hormones by antiidiotypes

It has been shown that anti-anti-X can resemble X, not only in shape, but also in function for the case that X is a hormone. This has been shown using monoclonal antibodies and antiidiotypic antibodies to the hormone alprenolol. The function of alprenolol is to bind to the adrenergic receptor and stimulate adenyl cyclase activity. Some anti-anti-alprenolol monoclonal antibodies resemble alprenolol sufficiently closely that they too bind to the adrenergic receptor and stimulate adenyl cyclase activity.[99,100]

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[99] S. Chamat, J. Hoebeke, A. D. Strosberg. (1984) Monoclonal antibodies specific for beta-adrenergic ligands. J. Immunol. 133, 1547-52.

Idiotypes of Ab-1, Ab-2, Ab-3 and Ab-4

Additional evidence of symmetry between idiotypes and antiidiotypes in stimulatory interactions was reported by Urbain et al.[101] They purified antibodies to an antigen, and following the Richter terminology called them Ab-1. They then raised antiidiotypic antibodies against Ab-1 to give Ab-2. Immunization with purified Ab-2 produced Ab-3, and immunization with Ab-3 was used to produce Ab-4. They found that Ab-4 reacted with both Ab-1 and Ab-3, just as Ab-2 did. This result is most simply interpreted in terms of symmetrical interactions between the even and odd numbered antibodies, so that Ab-3 has a similar idiotype to that of Ab-1 (as defined by Ab-2 and Ab-4) and Ab-4 has a similar idiotype to that of Ab-2 (as defined by Ab-3 and Ab-1).

Symmetry as the consequence of the cross-linking postulate

The idea that V-V stimulatory interactions are symmetrical is consistent with the concept that stimulation of lymphocytes involves the cross-linking of their specific receptors. If a V region "A" in bivalent or multivalent form is able to cross-link cell receptors with a V region "B", we can reasonably expect that the V region B in bivalent or multivalent form will be able to cross-link cell receptors with the V region A.

The cross-linking model for causing proliferation of B cells predicts that anti-immunoglobulin (anti-Ig) should be able to cause B cell proliferation. A review of published data revealed that of 36 studies, 32 investigators observed such proliferation (including one "sometimes") and 4 failed to do so.[102] In most of the cases of negative results, the experiments were done with antisera, not purified antibody. One of the more detailed studies was by Sieckmann et al.[103], which included experiments with purified anti-μ antibody (antibody specific for the heavy chain of IgM). They reported a case of a non-stimulatory anti-IgM antiserum from which they were able to prepare specific antibodies that were strongly stimulatory. The conclusion is that the inability of some

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[100] J. G. Guillet, S. V. Kaveri, O. Durieu, C. Delavier, J. Hoebeke, A. D. Strosberg. (1985) Beta-adrenergic agonist activity of a monoclonal anti-idiotypic antibody. Proc. Nat. Acad. Sci. (USA) 82, 1781-1784.

[101] J. Urbain, C. Colligno, J. D. Frassen, B. Mariame, O. Leo, G. Urbainva, M. Wikler and C. Wuilmart (1979) Ann. Immunol. (Paris) C130, 289-291.

[102] G. W. Hoffmann (1980) On network theory and H-2 restriction. Contemp. Topics in Immunobiol. 11, page 191.

[103] D. G. Sieckmann, R. Asofsky, D. E. Mosier, I. M. Zitron, W. E. Paul (1978) Activation of mouse lymphocytes by anti-immunoglobulin. I. Parameters of the proliferative response. J. Exp. Med. 147, 814-829.

anti-Ig antisera to stimulate proliferation is probably due to inhibitors in these antisera.

Symmetry in killing

In 1983 experiments by Anwyl Cooper-Willis in my laboratory showed that killing interactions between idiotypes and antiidiotypes are symmetrical.[104] This experiment involved two mutually specific IgM monoclonal antibodies (I will call them "A" and "B") that were attached to two sets of red blood cells (RBC). The RBC-A complexes were lysed by B antibodies and the RBC-B complexes were lysed by A antibodies. This was a very simple experiment that confirmed a basic postulate of the symmetrical network theory. In fact the theory predicted this result.[105]

First symmetry

Symmetry in idiotypic interactions is now well established. If an idiotype is recognized by a complementary antiidiotype, then the former is also an antiidiotype of the latter. This concept has been called "First Symmetry". When the evidence supporting symmetry is marshaled, one might ask, how could one have thought otherwise? The fact that the asymmetric view existed is due to the strong influence of the enzyme/substrate analogy on thinking about the interactions between V regions and antigens.

The alternative to the cross-linking model was that the binding of an antigen to a specific receptor would cause a change in shape ("conformational change") of the receptor, and that this change would be transmitted all the way through the membrane to the inside of the cell. Such a change would permit the receptors, acting independently of each other, to transmit information to the interior of the cell.

Apart from the evidence supporting cross-linking as the mechanism for the activation of lymphocytes, there are two main reasons for rejecting the conformational change model. The first is that the antibody has a thin, flexible region called the hinge region shown in **Figure 2-2**, between the (V_H, V_L, C_L, C_{H1}) part of the molecule and the (C_{H2}, C_{H3}) parts, and it is difficult to imagine the transmission of a conformational change across that region. R. Poljak, a crystallographer working on antibody structure has written in this context that "transmission of specific conformational signals from the

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[104] A. Cooper-Willis and G. W. Hoffmann. Symmetry of effector function in the immune system network. Molecular Immunology 20, 865-870, 1983.

[105] Confirmed prediction.

antibody combining site to C_{H2}, along a distance of 100 angstroms, is difficult to visualize."[106]

The second reason for rejecting the conformational change model is that it ascribes an unreasonable amount of "molecular intelligence" to molecules with V regions that are, to a large extent, generated by a random mutation process.[107] Conformational changes, that involve a particular function being generated at one site when a substrate binds at another site, are fancy molecular engineering. They normally work only if the substrate binds at the precisely defined site, and only a very limited class of substrates are capable of inducing the conformational change. Ig receptors can deliver an activating signal following the binding of antigen, antiidiotype antibodies, anti-allotype antibodies or anti-isotype antibodies, each of which binds to a different site. It is unreasonable to expect the same activating conformational change to follow from the binding of each of these reagents. On the other hand, each of them can cause cross-linking. Monovalent fragments of antigen and anti-Ig reagents should be able to induce conformational changes (if that were the mechanism of activation), but in fact, and in agreement with the cross-linking hypothesis, they are unable to cause activation.

There has been a widespread and deeply rooted belief among immunologists that the stimulation of T cells is fundamentally different from the stimulation of B cells. This is because the T cell repertoire is known to be biased towards the recognition of MHC molecules, as discussed here in chapters 12, 13 and 17. This bias does not however preclude that also T cells are stimulated by the cross-linking of their specific receptors. Indeed, monoclonal antibodies specific for the V regions of T cell receptors can be stimulatory for T cells[108], as can monoclonal antibodies specific for non-variable molecules called T3, that are non-covalently bound to the T cell receptor. The latter stimulation is analogous to the stimulation of B cells with anti-immunoglobulin. There are also reports of a monoclonal antibody that binds to the T cell receptor of approximately 20% of resting peripheral (non-thymic) T cells and induces proliferation in

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[106] R. Poljak (1978) Correlations between three-dimensional structure and function of immunoglobulins. Critical Reviews Biochem. 5 , 45-84.

[107] S. Tonegawa, N. Hozumi, G. Matthyssens and R. Schuller, (1976) Somatic changes in the content and context of immunoglobulin genes. Cold Spring Harbor Symposium on Quantitative Biology, 41, 877-889.

[108] J. Kaye, S. Porcelli, J. Tite, B. Jones and C. A. Janeway (1983) Both a monoclonal antibody and antisera specific for determinants unique to individual cloned helper T cell lines can substitute for antigen and antigen-presenting cells in the activation of T cells. J. Exp. Med. 158, 836-856.

these T cells.[109,110] Taken together, these results are consistent with the cross-linking of receptors being stimulatory, while not denying the role of the MHC in shaping the T cell repertoire. We will initially develop the basic aspects of the symmetrical network theory (chapters 10 and 11) within the simple model of T cells and B cells each being susceptible to stimulation by a very wide range of substances, that are able to cross-link their respective receptors.

In my opinion the cross-linking of receptors as the basis for stimulatory interactions in the immune system is one of the most fundamental facts about the immune system following the fact of clonal selection.

Symmetry in the blocking of receptors by antigen specific T cell factors

Antigen-specific T cell factors are molecules that are like Fab fragments of antibodies, but are secreted by T cells. On the basis of their molecular weight of approximately 50,000 daltons, they are believed to have only one V region, in contrast to IgG antibodies, that have a molecular weight of 150,000 and two V regions. For an antigen X, monovalent anti-X specific T cell factors in soluble form are assumed to be able to block antigenic determinants of X and the receptors of anti-anti-X cells, while anti-anti-X factors block the receptors of anti-X cells.

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[109] U. D. Staerz, H.-G. Rammensee, J. D. Benedetto and M. J. Bevan (1985) Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. J. Immunol. 134, 3994-4000.

[110] U. D. Staerz and M. J. Bevan (1986) Activation of resting T lymphocytes by a monoclonal antibody directed against an allotypic determinant on the T cell receptor. Eur. J. Immunol. 16, 263-270.