

## Chapter 14. Second symmetry

Tyger Tyger, burning bright  
In the forests of the night  
What immortal hand or eye  
Could frame thy fearful symmetry?  
-William Blake  
Songs of Experience (1794)

A good general rule is to state that  
the bouquet is better than the taste  
and vice versa.  
-Stephen Potter  
One-Upmanship (1952)

### Anti-foreign and anti-anti-self in allo-antisera

Lymphocytes can be used as experimental antigens. Immunizations with foreign lymphocytes have played a central role in the development of immunogenetics. MHC molecules are important antigens on lymphocyte surfaces. When a mouse is immunized with lymphocytes of a strain that has a different MHC haplotype, the resulting immune serum is called an allo-antiserum. The frequency of cells that respond to foreign MHC on lymphocytes is high compared with the frequency of cells that respond to other antigens, such as soluble protein antigens. This can be understood in terms of the selection of T cells to recognize self MHC molecules. Up to a few percent of the cells that have been selected to recognize self MHC also recognize a particular foreign MHC molecule, since a foreign MHC molecule is not very different from self MHC.

However, foreign lymphocytes injected into an animal (a "recipient") have, in addition to MHC, another important antigenic entity on their surfaces. These are the specific receptors of the foreign lymphocytes that recognize antigens of the recipient. The foreign lymphocytes with these receptors are stimulated to proliferate by the recipient antigens, and these receptors have V regions that are immunogenic in the recipient. The most important of these foreign lymphocytes are the ones with receptors that recognize the recipient's MHC.

If we immunize a strain A animal with strain B lymphocytes, the immune response of the A animal thus has two components, namely A anti-B<sup>209</sup> and

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<sup>209</sup> Antibodies made by an A mouse against B strain antigens

A anti-(B anti-A)<sup>210</sup>. We will also call these more simply anti-B (or anti-foreign) and anti-anti-A (or anti-anti-self). The converse immunization of strain A with strain B lymphocytes induces the production of anti-A and anti-anti-B. The response in the A animal is thus complementary to the response in the B animal. Anti-B in the first response is complementary to the anti-anti-B in the second response, while anti-A in the second response is complementary to the anti-anti-A in the first response. This is known as "second symmetry".

### Experimental validation

Ramseier and Lindenmann<sup>211</sup> and Binz and Wigzell<sup>212</sup> were the first to study anti-anti-self antibodies raised by alloimmunization. Their work mainly involved immunizing F1 animals (say AxB) with parental cells (say strain A). The AxB animals could not respond to A strain antigens, which are self antigens, but could respond to the receptors on A strain lymphocytes that recognise B strain alloantigens. The AxB animals therefore made an anti-anti-B response that could be shown to react with anti-B antibodies.

My colleagues and I formulated second symmetry as a more general principle, and validated it initially by showing that an A anti-B alloimmune serum can be used to specifically inhibit antibody plus complement mediated killing by the converse B anti-A serum and vice versa.<sup>213</sup> In a second study anti-anti-self activity was demonstrated in column purified IgG obtained from alloimmune sera.<sup>214</sup> In that study we also demonstrated the inhibition of B anti-A cytotoxic T cells by A anti-B serum and vice versa.

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<sup>210</sup> Antibodies made by an A mouse against B anti-A antibodies or against B anti-A specific receptors.

<sup>211</sup> H. Ramseier and J. Lindenmann (1972) Aliotypic antibodies. *Transplantation Reviews*, 10, 57-96.

<sup>212</sup> H. Binz and H Wigzell (1975) Shared idiotypic determinants on B lymphocytes and T lymphocytes reactive against the same antigenic determinants. I. Demonstration of similar or identical idiotypes on IgG molecules and T cell receptors for alloantigens. *J. Exp. Med.* 142, 197-211.

<sup>213</sup> G. W. Hoffmann, A. Cooper-Willis and M. Chow (1986) A new symmetry: A anti-B is anti-(B anti-A), and reverse enhancement. *J. Immunol.*, 137, 61-68.

<sup>214</sup> A. Al-Fahim and G. W. Hoffmann (1998) Second symmetry in the Balb/c-C57BL/6 (B6) system, and anti-anti-self antibodies in aging B6 mice. *Immunol. and Cell Biol.* 76, 27-33.

### Similarity in the idiotypes of B cells and T cells specific for alloantigens

The work by Binz and Wigzell led to the conclusion that the same idiotypes are expressed by T cells and B cells that are specific for alloantigens of a given strain.<sup>212</sup> Our work on second symmetry supports this, in the sense that anti-anti-self antibodies in an A anti-B serum both bind to anti-foreign antibodies of a B anti-A serum and interact with anti-foreign T cells of a B anti-A immune animal. Different genes are used to encode T cell receptors and B cell receptors, but the idiotypes of anti-MHC T cell receptors and anti-MHC antibodies are similar, as defined serologically by anti-anti-self antibodies.

### Activity in an A anti-B serum absorbed with B

A anti-B serum or purified antibodies can be absorbed against the immunizing B strain cells without loss of the specific inhibitory effect.<sup>213,214</sup> This absorption removes anti-B but not anti-anti-A antibodies, and thus permits these two components of alloimmune serum to be studied independently. Anti-B antibodies were previously known to be able to block strain B antigens, and hence enhance the survival of a B strain graft in an A strain animal.<sup>215,216,217</sup> We found that the anti-anti-A antibodies in an alloimmune, immunogen absorbed, A anti-B serum are also able to prolong the survival of an A strain graft on a B strain animal, a phenomenon we called reverse enhancement.

The fact that an absorbed serum (A anti-B, absorbed with B) has any activity at all may appear paradoxical from a non-network viewpoint. Absorption against the antigen (B strain lymphocytes) might have been expected to remove all the specific activity. That is, one might ask why the anti-A receptors on the B strain lymphocytes would not have absorbed all the anti-anti-A activity as well. There are two considerations that together may account for this. Under the conditions of the absorption experiment, there may firstly be more anti-anti-A antibodies in the A anti-B serum than anti-A receptors in the absorbing cells. Likewise, under the conditions of the experiment, there may also be more B strain alloantigens on the B strain cells than anti-B antibodies in the serum. The fact that anti-B is removed while anti-anti-A is not removed indicates that both of these possibilities may apply.

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<sup>215</sup> C. B. Carpenter, A. H. F. d'Apice and A. K. Abbas (1976) The role of antibodies and in the rejection of organ allografts by alloantibody. *Adv. Immunol.* 22, 1-

<sup>216</sup> G. A. Voisin (1980) Role of antibody classes in the facilitation reaction. *Immunol. Rev.* 49, 3-59

<sup>217</sup> P. J. Morris (1980) Suppression of rejection of organ allografts by alloantibody. *Immunol. Rev.* 49, 93-125.

### Enhanced graft survival ascribed to anti-anti-self antibodies

In the reverse enhancement experiment three doses of just five microliters of alloimmune, immunogen absorbed serum (A anti-B, absorbed B) were found to be able to specifically prolong the survival of a graft of skin from an A strain animal onto a B strain recipient.<sup>213</sup> The serum contained anti-anti-A antibodies, and the injections were given on days -4, -1 and 0 relative to grafting. Normally the B strain animal mounts an anti-A immune response against the graft. The prolongation of the graft survival may be ascribed to anti-anti-A antibodies in the absorbed serum inhibiting (blocking) the receptors of anti-A clones in the B strain host. It may alternatively be ascribed to the anti-anti-A antibodies stimulating the host immune system with respect to a shape space axis defined by anti-A and anti-anti-A, perturbing the system towards the symmetrical suppressed state for these two specificities. The very small amount of serum needed suggests that the latter, more active mechanism applies. If this is the case, more doses of the alloimmune, immunogen absorbed serum prior to and/or following grafting should result in a more profound prolongation of the graft survival.<sup>218</sup>

### Anti-anti-self antibodies in an ELISA assay

Second symmetry interactions can also be demonstrated in a simple ELISA assay.<sup>219</sup> The ELISA assay used is that of Figure 3-1, with (i) normal serum, (ii) alloimmune (A anti-B) serum, or (iii) alloimmune, immunogen absorbed (A anti-B absorbed with B) serum on the plate, and biotinylated B anti-A serum being the next step. Specifically, biotinylated Balb/c anti-B6 antiserum binds to B6 anti-Balb/c serum, to B6 anti-Balb/c serum that has been absorbed with the immunogen, but not to normal B6 serum, nor normal Balb/c serum, nor Balb/c anti-B6 serum, nor Balb/c anti-B6 serum absorbed with the immunogen.<sup>219</sup> Conversely, biotinylated B6 anti-Balb/c antiserum binds to Balb/c anti-B6 serum and to Balb/c anti-B6 serum that has been absorbed with the immunogen, but not to normal B6 serum, nor normal Balb/c serum, nor B6 anti-Balb/c serum, nor Balb/c anti-B6 serum absorbed with the immunogen. Qualitatively similar results were obtained using column purified IgG.<sup>220</sup> These are criss-cross specificity controlled experiments, in which in

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<sup>218</sup> Prediction

<sup>219</sup> T. A. Kion, Ph. D. thesis, University of British Columbia, 1991.

<sup>220</sup> A. Al-Fahim and G. W. Hoffmann (1998) Second symmetry in the Balb/c-C57BL/6 (B6) system, and anti-anti-self antibodies in aging B6 mice. *Immunol. and Cell Biol.* 76, 27-33.

one case the biotinylated serum is Balb/c anti-B6, and in the other case the biotinylated serum is B6 anti-Balb/c.

### **Summary of assays for the demonstration of anti-anti-self antibodies**

To summarize, the following assays have been used to demonstrate second symmetry:

- inhibition of antibody mediated cytotoxicity (antibodies inhibit antibodies)
- inhibition of graft rejection (antibodies inhibit or stimulate T cells with complementary specificity)
- the ELISA assay (antibodies bind to antibodies)
- inhibition of cellular cytotoxicity (antibodies inhibit killing by T cells)

The immunization of strain A with strain B cells involves two living sets of cells, the A strain host and the B strain foreign inoculum or "graft", and there are a total of four immune responses that we need to consider, two by the graft and two by the host. These are:

- host versus graft MHC (A anti-B);
- graft versus host MHC (B anti-A);
- host versus the receptors of the graft that have anti-host specificity (anti-anti-A); and
- graft versus the receptors of the host that have anti-graft specificity (anti-anti-B).

The two host responses are stronger than the two graft responses (in a healthy host), and the graft cells are eliminated, leaving us with only the host anti-B and the host anti-anti-A immunity. The fact that strain B cells can absorb anti-B antibodies but not anti-anti-A antibodies implies that the ratio of strain B antigen on the cells to anti-B antibodies in the serum is greater than the ratio of anti-A receptors on B strain cells to anti-anti-A antibodies in the serum. This suggests that the graft anti-A receptors are a highly efficient antigen, in the sense of a small amount of antigen causing a strong immune response. This would not be surprising, since anti-host lymphocytes injected into a host would be stimulated to proliferate before being eliminated by the host anti-graft response.

Second symmetry demonstrates, simply and in a compelling fashion, that antibodies can be made in vivo to the V regions of the receptors of lymphocytes. The repertoire of specificities of antibodies includes the repertoire of V regions that recognize alloantigens.

### **Extended second symmetry: anti-foreign, anti-anti-self and anti-I-J**

Second symmetry can be taken one step further in light of our interpretation of the I-J phenomenon. We know that alloimmunizations induce the production of anti-I-J antibodies, and we interpret I-J to be an internal

image of the repertoire of self antigens, especially self MHC class II. I-J is anti-anti-self MHC class II or an image of self MHC class II, and anti-I-J is then anti-(MHC class II image), at least to a first approximation. (Other self antigens, including especially MHC class I, may also impact to some extent on the I-J centre-pole.) The relationships between MHC, anti-MHC, MHC image (anti-anti-self) and anti-MHC image (anti-I-J) in an A anti-B serum and a B anti-A serum are shown in Figure 14-1. With the inclusion of I-J and anti-I-J, the total A anti-B response is again seen to be complementary to the total B anti-A response. This is called extended second symmetry.

The antigens in a preparation of strain A cells injected into a strain B animal and the antibodies they induce are as follows:

1. Self A antigens that differ from those of strain B antigens (alloantigens). These induce an anti-A (anti-foreign, "B $\alpha$ A") response in a strain B animal.
2. Strain A lymphocyte receptors that recognize strain B alloantigens. These have  $\alpha$ B V regions and they induce an anti-anti-B (anti-anti-self, "B $\alpha\alpha$ B") response the strain B animal.
3. The strain A centre-pole (I-J<sup>A</sup>), which in our theory is  $\alpha\alpha$ A. These induce a B $\alpha\alpha\alpha$ A response in strain B which is anti-anti-anti-A or anti-anti-anti-foreign. These are the anti-I-J<sup>A</sup> antibodies.

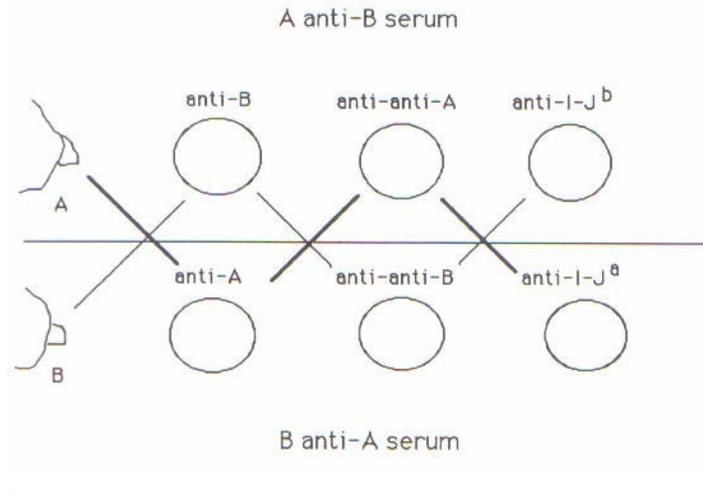
The corresponding antigens in the converse immunization of B strain cells injected into an A strain animal are the B alloantigens, strain B lymphocyte receptors that recognise A alloantigens, and I-J<sup>B</sup>. The antibodies they induce are  $\Lambda\alpha$ B,  $\Lambda\alpha\alpha$ A and B $\alpha\alpha\alpha$ A (anti-I-J<sup>B</sup>). Again the complete set of antibodies in the anti-A response is complementary to the complete set of antibodies in the anti-B response. This theory predicts that  $\alpha$ I-J antibodies in one alloimmune serum can be shown to bind specifically to  $\alpha\alpha$ self antibodies in the converse alloimmune serum.<sup>221</sup>

The fact that the B $\alpha$ A antibodies are made in a B strain mouse against A strain alloantigens rather than in, say, a C strain mouse, is important. Being made in a strain B mouse ensures that the antibodies have no activity against B strain antigens, assuming (as is normally the case) that self tolerance has not been broken in the course of the alloimmunizations. The B $\alpha$ A antibodies are both  $\alpha$ A and "not  $\alpha$ B". The latter is an essential aspect of these antibodies, and this restriction is assured by the presence in the serum of the B $\alpha\alpha$ B antibodies.

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<sup>221</sup> Prediction

Figure 14-1. Extended second symmetry. A model of the three classes of antibodies in two complementary alloimmune sera, A anti-B and B anti-A. Each component in one serum is complementary to one or more components in the other serum. This model predicts that the anti-I-J antibodies in each of the alloantisera bind to ant-anti-self antibodies in the converse antiserum.



Denoting the  $\alpha A$  antibodies made in a B animal as  $B\alpha A$ , rather than just  $\alpha A$  emphasizes the identity of the mouse that makes these allo-antibodies, and is an important aspect of their specificity. The same applies to denoting the  $\alpha\alpha B$  and  $\alpha\alpha\alpha A$  antibodies made in the same immunization (in the B animal immunized with A cells) as  $B\alpha\alpha B$  and  $B\alpha\alpha\alpha A$ . The corresponding antibodies in the converse serum are  $A\alpha B$ ,  $A\alpha\alpha A$  and  $A\alpha\alpha\alpha B$ .

### Second symmetry and shape space

Shape space is a concept in which similar shapes map closely to each other in a space of some dimensionality, and the more different two shapes are, the further they are from each other in the space.<sup>222,223</sup> Two complementary substances, or two groups of complementary substances, or one substance and a complementary group of substances can each be used to define an axis in

<sup>222</sup> A. S. Perelson and G. Oster (1979) Theoretical studies of clonal selection: minimal antibody repertoire size and reliability of self-nonself discrimination. *J. theor. Biol.* 81, 645-667.

<sup>223</sup> A. Lapedes and R. Farber (2001) The geometry of shape space: application to influenza. *J. theor. Biol.* 212, 57-69.

shape space<sup>224</sup>. Since  $B\alpha A$  is complementary to  $A\alpha\alpha A$  these two sets of antibodies can be used to define an axis in shape space, as shown in Figure 14-2. The  $\alpha I\text{-J}^A$  antibodies are  $B\alpha\alpha\alpha A$  and are complementary to  $A\alpha\alpha A$ , so they map on the left side of this shape space axis. The conventional A strain alloantigens (“A”) are complementary to  $B\alpha A$ , so they map on the right side of the axis.

The fact that A and  $B\alpha\alpha\alpha A$  are on the opposite sides of this shape space axis does not imply that  $B\alpha\alpha\alpha A$  should bind to the conventional A alloantigens. Indeed, there is no experimental evidence that they do so. (This can be seen as a matter of definition. Antibodies from an animal B immunized with A strain cells, that bind to conventional A alloantigens, are regarded as  $B\alpha A$  rather than  $B\alpha\alpha\alpha A$ .) The key point is that both A and  $B\alpha\alpha\alpha A$  can be mapped using the defining antibodies for the axis, namely  $B\alpha A$  and  $A\alpha\alpha A$ , independently of any possible binding to each other.

The next question is, where would we expect  $B\alpha\alpha B$  map on this axis? One way of looking at it is to say that  $A\alpha\alpha A$  and  $B\alpha\alpha B$  are both  $\alpha\alpha$ self, so they may be similar to each other, and therefore may map on the same side as each other. On the other hand  $B\alpha\alpha B$  is similar to both  $B\alpha A$  and  $B\alpha\alpha\alpha A$  in that they are all not  $\alpha B$ , so we could conclude that  $B\alpha\alpha B$  should map on the left side of the axis.

A solution to this question may lie in defining a separate shape space axis on which to map  $\alpha\alpha B(B)$ . This axis would be defined by  $A\alpha B$  and  $B\alpha\alpha B$ , and we can most simply draw it as shown in Figure 14-3, namely orthogonally to the  $B\alpha A\text{-}\alpha\alpha\alpha A(A)$  axis. The  $B\alpha\alpha B$  antibodies could thus be a defining point for the second axis. The B alloantigens and  $A\alpha\alpha\alpha B$  (that is,  $\alpha I\text{-J}^B$ ) would map on the second axis as shown.

Another way of looking at this is that there is no reason that either  $B\alpha\alpha B$  or  $A\alpha B$  should react strongly with  $B\alpha A$  or  $A\alpha\alpha A$ , while they do react strongly with each other. Hence when we measure the binding of  $B\alpha\alpha B$  and  $A\alpha B$  to  $B\alpha A$  and  $A\alpha\alpha A$  to map them on the  $B\alpha A\text{-}A\alpha\alpha A$  axis, we can expect to obtain coordinates of approximately  $0 - 0 \approx 0$  for each of them, in contrast to (with appropriate normalization) 1 for  $B\alpha\alpha B$  and -1 for  $A\alpha B$  on the  $B\alpha\alpha B\text{-}A\alpha B$  axis, as shown in Figure 14-3. Hence the two axes are expected to be at least approximately orthogonal to each other.

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<sup>224</sup> G. W. Hoffmann (2003) Proteomic Analyser with Applications to Diagnostics and Vaccines. *J. theoret. Biol.* 228, 459-465.

Figure 14-2. The anti-foreign antibodies in a B anti-A alloimmune serum, denoted  $B\alpha A$ , together with the anti-anti-self antibodies in the converse A anti-B serum, denoted  $A\alpha\alpha A$ , define an axis in shape space. The anti-I-J antibodies in the B anti-A alloimmune serum, are postulated to be complementary to  $A\alpha\alpha A$ . They are denoted as  $B\alpha\alpha\alpha A$ , and map on the left side of this axis. A strain MHC antigens are complementary to  $B\alpha\alpha A$  antibodies and therefore map on the right side of this axis.

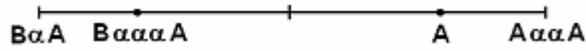
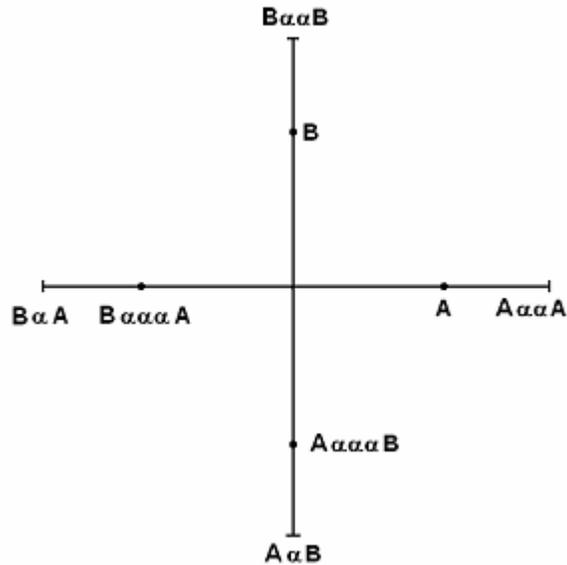


Figure 14-3. The anti-foreign antibodies  $A\alpha B$  and the anti-anti-self antibodies  $B\alpha\alpha B$  define a second axis in shape space that is drawn orthogonal to the  $\alpha A-\alpha\alpha A$  axis, since the binding of  $A\alpha B$  and  $B\alpha\alpha B$  antibodies to  $B\alpha A$  and  $A\alpha\alpha A$  antibodies can be expected to be small compared with their binding to each other.



With the same normalization for both axes, we would then have defined the coordinates of the four reagents that are the basis of the two-dimensional shape space thus:

Reagent	Coordinates
B $\alpha$ A	(-1, 0)
A $\alpha\alpha$ A	(1, 0)
A $\alpha$ B	(0, -1)
B $\alpha\alpha$ B	(0, 1)

It follows that the coordinates of the alloantigens A, B and the antibody populations  $\alpha\alpha\alpha$ A(B) and  $\alpha\alpha\alpha$ B(A) are predicted in this model to have the values

A	(>0, 0)
B	(0, >0)
B $\alpha\alpha\alpha$ A	(<0, 0)
A $\alpha\alpha\alpha$ B	(0, <0)

as shown in Figure 14-3. In principle these coordinates can be experimentally determined by measuring the binding of the four reagents, that are the basis of the two-dimensional shape space, to each of A, B, A $\alpha\alpha\alpha$ B and B $\alpha\alpha\alpha$ A.