

## Chapter 6. Regulation: T cells and B cells

The Governor was strong upon  
The Regulations Act  
The Doctor said that death was but  
a scientific fact  
And twice a day the Chaplain called,  
and left a little tract  
-Oscar Wilde  
The Ballad of Reading Gaol (1898)

The problems of knowing when the immune system will respond to an antigen by producing antibodies and when not, how the system responds, and why, and which cells live and which ones die, are problems of immune system regulation. The aim of much research in cellular immunology has been to understand immune regulation in terms of network theory.

Prior to beginning our development of network theory in chapter 8, we will look at some of the system response phenomenology that especially pointed toward network theory as the appropriate paradigm. This is the "top-down" approach we mentioned in chapter 1. We start with learning more about known modes of response of the system, and then (in later chapters) seek the simplest model, consisting of known and/or plausible components, that could give rise to the observed phenomena.

The late 1960s to early 1980s, was a time of especially rapid progress in cellular immunology. Each issue of the major journals brought the latest news from the many laboratories engaged in the detective work. Helper T cells, suppressor T cells and contrasuppressor cells were discovered. Research was directed towards trying to understand how T cells and B cells cooperate in the production of antibodies, towards understanding the phenomenon of tolerance, and trying to see how the puzzling phenomenon of suppression fits into the overall picture. The focus was especially on antigen-specific regulatory mechanisms.

### Living test tubes

Experiments designed to study immune regulation frequently involve firstly the preparation of various defined populations of mouse lymphocytes, and then injecting these cells into mice that function as living test tubes for investigating interactions between the cells. The mice into which the lymphocytes are injected are called the "recipients", and are irradiated with X-rays. This prevents their own lymphocytes from proliferating and participating in the response. The immune response observed then depends only on the injected cells. The entire process is called an "adoptive transfer" experiment, since the lymphocytes are transferred to a new host, which

adopts them as its own. The response of the injected lymphocytes can then be studied in a controlled fashion.

### Helper T cells

An adoptive transfer experiment was performed in 1966 by Claman and his colleagues,<sup>36</sup> in which they showed that T cells help B cells to make antibodies. The experiment is illustrated in Figure 6-1. Thymus cells (T cells) and bone marrow cells (mainly B cells) are mixed and injected together with an antigen into an irradiated recipient. After a week, this recipient had many more cells in its spleen making antibody to the antigen than either of two control groups of animals, one of which received only T cells and the other of which received only B cells. The antibody response was detected using the plaque assay described in chapter 3.

### B cells make antibodies

A second experiment, illustrated in Figure 6-2, showed that B cells are the ones that make antibody, while T cells have a "helper" role.<sup>37</sup> The experiment was similar to the preceding one, but in this case the T cells and B cells were obtained from mice of two different strains, X and Y. Prior to the plaque assay, the spleen cells from the irradiated recipient were treated with either anti-X or anti-Y antibodies, in a way that killed either all the T cells or all the B cells, leaving one population consisting of only B cells and the other of only T cells. Plaques were found to be made by the B cell population but not by the T cells, so the B cells are the ones that make and secrete antibody.

### Haptens and carriers

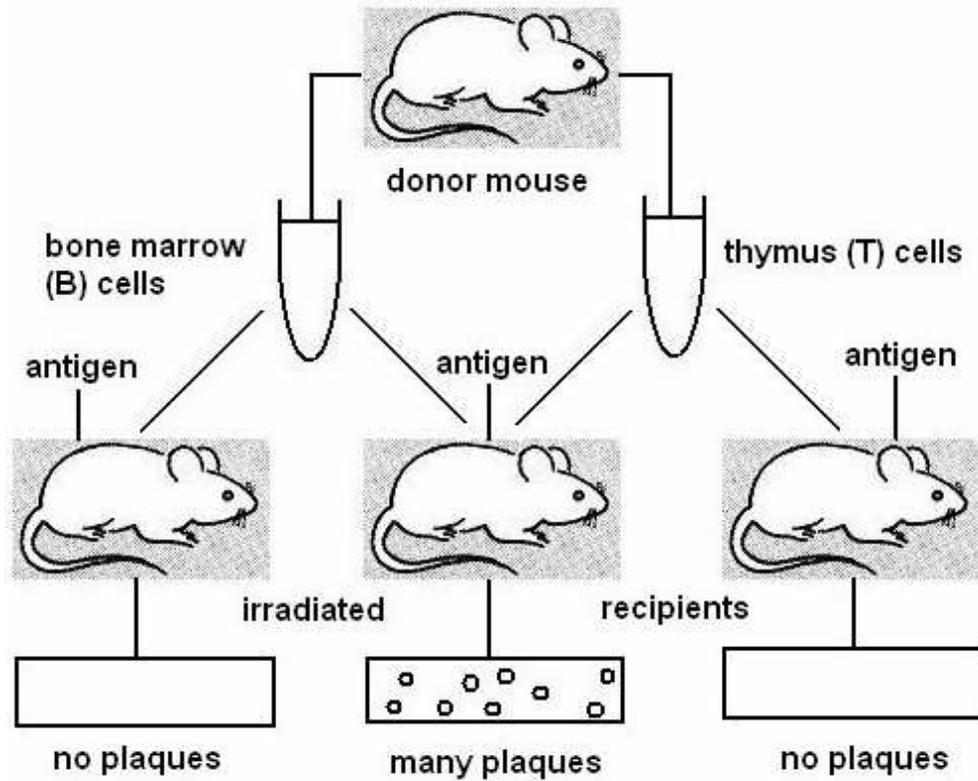
How big does a substance have to be in order to evoke an immune response? Saline solution injected into an animal does not induce an immune response, suggesting that sodium and chloride ions do not function as antigens. We can obtain immune responses to quite small molecules and functional groups called "haptens", such as dinitrophenyl ("DNP"), 4-hydroxy-3-nitrophenyl acetyl ("NP"), and trinitrophenyl ("TNP"). Haptens are however much smaller than proteins, and they mostly have to be coupled to a larger molecule such as a protein or a polysaccharide to function as an antigen. The larger molecule is then called a "carrier." In the early days of studying the immune response to hapten-carrier combinations, it was found that B cells make

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<sup>36</sup> H. N. Claman, Chaperon, E. A., and Triplett, R. F. (1966) Immunocompetence of transferred thymus-marrow cell combinations. *J. Immunol.* 97, 928-832.

<sup>37</sup> G. F. Mitchell and J. F. A. P. Miller (1968) Cell to cell interactions in the immune response. II. The source of hemolysin forming cells. *J. Exp. Med.* 128, 821-837.

Figure 6-1 The experiment by Claman et alia that demonstrated that both B cells and T cells are needed in order to make an immune response. The B cells were bone marrow cells and the T cells were thymus cells. Recipient mice were irradiated to deplete them of their own lymphocytes, and reconstituted with only B cells, both B cells and T cells, or only T cells. Only the mice that received both B cells and T cells responded to the antigen, as measured in an antigen-specific plaque forming assay.



antibodies mainly in response to the hapten, while the carrier evoked mainly T cell immunity. Injection of a mouse with the hapten DNP coupled to the protein keyhole limpet hemocyanin ("DNP-KLH") induces the production of antibodies to DNP, and normally also to KLH. If we are interested only in the anti-DNP antibody response, we can for example determine the number of plaque forming cells in the spleens of immunized mice using DNP coupled to sheep red blood cells.

### Antigenic competition

The immune system typically is able to make a strong response to a single antigen, but responds poorly to a second antigen given soon after a first antigen, whereby the second antigen may be unrelated to the first.<sup>38</sup> This phenomenon, called antigenic competition, has also been observed in the induction of specific tolerance. These findings are paradoxical within the simple clonal selection concept in which the clones for different antigens are independent entities, each responding to its own antigen, regardless of what happens to others. This was one of the phenomena that was originally cited by Jerne to support the immune network hypothesis, in which clones are assumed to be linked to each other via V-V interactions. He reasoned that a perturbation to the entire network caused by the first antigen somehow inhibited the normal response of the network as a whole to the second antigen.

### T cells and tolerance

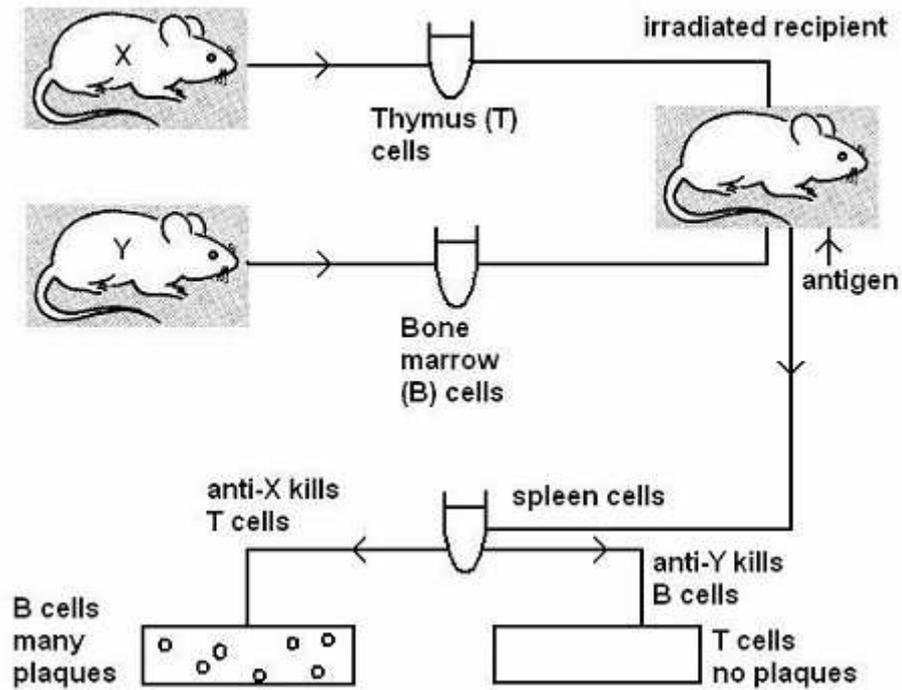
As mentioned in chapter 4, the injection of a foreign antigen does not always lead to an animal becoming more responsive to a second injection of the same antigen. The first injection can instead cause the animal to become unresponsive ("tolerant") with respect to the antigen. We saw that whether the antigen immunizes or tolerizes the animal depends on many parameters, including the route of injection, the dose (too little or too much can cause unresponsiveness) and the physical form of the antigen. Antigens can be *immunogenic* or *tolerogenic*. For example, aggregated proteins tend to cause an immune response, while deaggregated proteins (no clumps) tend to induce unresponsiveness. In the symmetrical network theory the difference is attributed to the aggregates being able to cross-link cell-surface receptors more effectively than deaggregated material, that lacks multiple identical determinants.

An obvious possibility would be that tolerance is due to the antigen-specific cells somehow being eliminated. This idea is however excluded by the experimental finding that antigen-reactive cells can be found in animals specifically tolerant for the antigen. This is true both for foreign antigens and for at least some self antigens, as discussed later.<sup>52,53,54</sup> In many cases it has been found that the key to unresponsiveness lies with the T cells.

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<sup>38</sup> J. Radovich and D. W. Talmadge (1967) *Science* 158, 512-514.

Figure 6-2 An experiment by Miller and Mitchell that demonstrated that B cells are responsible for making antibodies, while T cells have a helper function. Mice of two strains, here designated X and Y, were used. Thymus (T) cells from the strain X and bone marrow (B) cells from strain Y were combined in an irradiated recipient, that was also injected with an antigen. The latter mouse responded to the antigen, and when the cells making the response were treated with either anti-X plus complement to kill off the T cells or anti-Y plus complement to kill off the B cells, the antibody making cells were found in a plaque assay to reside in the B cell population.



### Suppressor T cells

The system response data that historically led most directly to network thinking is the phenomenon called suppression. Certain T cells, called suppressor T cells, when mixed with other T cells or B cells or both, are able to suppress an immune response. They are able to do this in a highly specific fashion. For example, it is possible to prepare a population of suppressor T cells that suppress only the immune response to a particular antigen X, and have no effect on the immune response to another antigen Y, even if Y is physically similar to X (say a similar protein antigen). A typical experiment that demonstrates the action of suppressor T cells is shown in Figure 6-3.

### Carrier-specific suppressor T cells

As noted above, in many experimental systems carrier primed T cells and hapten primed B cells have been shown to be able to combine with each other to give hapten-specific immune responses to the hapten-carrier conjugate. If however carrier primed T cells are combined with naïve T cells in an irradiated recipient, the carrier primed T cells can suppress the helper effect that the naïve T cells would otherwise exert. The carrier primed T cells are then effectively carrier specific suppressor T cells, and have a suppressive role on the response to the hapten of the hapten-carrier conjugate. An experiment by Tada and Takemori was done in 1974 which rigorously demonstrates the existence of carrier-specific suppressor T cells.<sup>39</sup> Their experiment involved the protein carriers keyhole limpet hemocyanin (KLH), bovine gamma globulin (BGG) and the hapten dinitrophenyl (DNP). Mice were immunized with 100 µg of the carriers KLH or BGG without adjuvant twice at two-week intervals. Two weeks after the second immunization spleen cells or thymocytes from these mice were injected into recipient mice that also received DNP-KLH or DNP-BGG with adjuvant. The results are shown in Table 6-1. The effect of the carrier-primed cells is to cause a profound, antigen(carrier)-specific suppression of the response to the hapten. The carrier primed cells not only fail to help an immune response to the hapten, they also prevent normal cells in the recipients from responding. The experiment included criss-cross specificity controls for the suppressive effect of thymocytes. KLH-primed cells suppress the response to DNP-KLH but not DNP-BGG, while BGG-primed cells suppress the response to DNP-BGG but not DNP-KLH. The authors also showed that the suppressor cells present in spleens are T cells. Specific suppression can also be demonstrated with *in vitro* experiments, as discussed later.

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<sup>39</sup> T. Tada and T. Takemori (1974) Selective roles of thymus-derived lymphocytes in the antibody response. I. Differential suppressive effect of carrier-primed T cells on hapten-specific IgM and IgG antibody responses. *J. Exp. Med.* 140, 239-252.

Table 6-1 Suppression of hapten-specific IgG antibody responses by carrier-primed thymocytes and spleen cells. Adapted from Tada and Takemori.<sup>39</sup>

Immunizing antigen	Cells transferred	IgG PFC/spleen on day 6
DNP-KLH plus adjuvant	None	11,000
	KLH-primed thymocytes	500
	KLH-primed spleen	89
	BGG-primed thymocytes	14,000
	BGG-primed spleen	11,600
DNP-BGG plus adjuvant	None	10,200
	BGG-primed thymocytes	287
	KLH-primed thymocytes	9,840

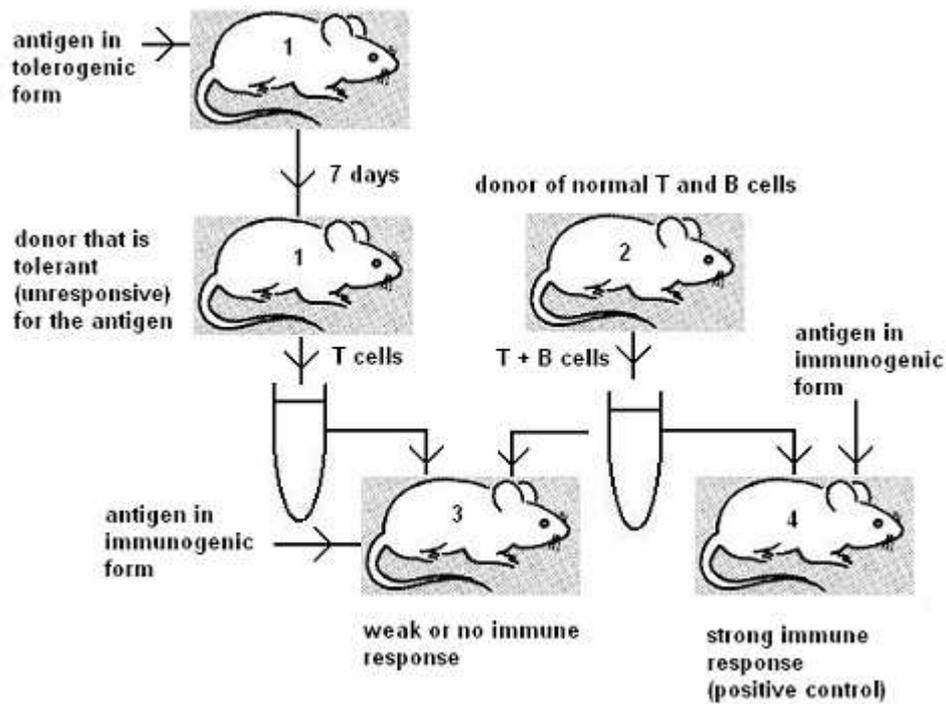
### The distinction between tolerance and suppression

The phenomenon of suppression is distinct from tolerance. If an animal has been treated with an antigen X in such a way that it is unable to respond to X given in a way that is normally immunogenic, we say that the animal has "been tolerized" or "is tolerant" or "is unresponsive" to X. We speak of suppression only if it is possible to obtain T cells from the tolerized animals that, when mixed with lymphocytes from a normal animal, are able to suppress an immune response of those normal cells to X. The induction of suppressor T cells does not necessarily involve a tolerogenic form of the antigen, nor is antigen-specific tolerance always associated with the presence of suppressor T cells as defined by the above type of experiment. There are many examples of tolerance without evidence of suppressor T cells. A challenge for a theory is to account for both the cases of specific unresponsiveness in which suppression can be demonstrated, and the cases in which no suppression can be demonstrated.

### Suppression and the origin of immune network theory

Network theory was originally formulated with the phenomenon of suppression very much in mind, as we will see in chapter 8. Antigen-specific suppressor T cells are simply explained in the context of the symmetrical network theory (chapter 10), and are not readily explained by competing theories. One set of cells specifically prevent another set of cells from responding to an antigen. In order to do this, the suppressing population has to be able to distinguish between the cells they suppress and other cells of the same kind but a different specificity. The most obvious way in

Figure 6-3 An experiment that demonstrates the generation and action of suppressor T cells. Mouse 1 is made unresponsive to an antigen by injecting it with an antigen in a tolerogenic form, for example a protein that has been made free of aggregates by ultracentrifugation. (Aggregated protein goes to the bottom of the centrifuge tube, while aggregate-free material remains in solution.) After 7 days mouse 1 is tolerant with respect to that antigen, and is a source of antigen-specific suppressor cells. Mouse 2 is a source of normal T cells and B cells. Mouse 3 and mouse 4 are irradiated to functionally delete their own lymphocytes. Mouse 4 receives the normal T cells and normal B cells from mouse 2, together with the antigen in immunogenic form, and makes a strong immune response. In addition to the normal T and normal B cells, mouse 3 is given cells from the mouse that has been made unresponsive with respect to the antigen. In spite of having the T and B cells needed to respond, mouse 3 fails to do so. The response of the normal cells is suppressed by tolerant T cells, that are therefore called suppressor T cells.



which they can make such a distinction is by using an interaction between their own V regions and the V regions of the suppressed population. This implies V-V interactions, that is, network interactions.

### **Suppression of V region or C region defined sets of B cells**

So far we have seen how we can induce T cells that suppress an immune response by B cells specific for a particular antigen. It is also possible to induce T cells that suppress antibody production by B cells defined by any one of a variety of epitopes on the V regions or C regions of the antibody receptors of the B cell. The epitope can be an idiotype (present on a small fraction of B cells), an allotype (present on approximately 50% of B cells of a given isotype in an F1 animal<sup>40</sup>), or an isotype (for example all the B cells expressing IgM receptors). We thus have several kinds of suppressor T cells, that are distinguished by having different specificities, but which are sufficiently similar to each other that they probably use the same mechanism.

### **Idiotype-specific tolerance**

In 1972 a new way for making a mouse tolerant to a specific antigen, phosphoryl choline ("PC"), was discovered by Cosenza and Köhler.<sup>41</sup> Antibodies were raised against a myeloma antibody that had anti-PC specificity. When these anti-anti-PC ("antiidiotypic") antibodies were injected into another mouse, that mouse became tolerant for PC. That is, the mouse was unable to make anti-PC antibodies when immunized with PC on a suitable carrier. This was a dramatic result because the only method for inducing specific tolerance to an antigen had previously been to use the antigen itself. Now it became apparent that not only was it possible for animals to make anti-antibodies, but these anti-antibodies can have profound regulatory effects.

### **Idiotype-specific suppression**

The starting point of immune network theory is the idea that the V region of an antibody is also an antigen. There is no reason why we should not be able to induce both specific immunity and specific tolerance to the V region of an antibody. We can also induce specific suppression for the production of a particular V-region. This is called "idiotype-specific" suppression.

The literature is not consistent in the use of the term "suppression" with respect to idiotypes. Simply the inability to produce a given idiotype (in response to a

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<sup>40</sup> A cross between two strains A and B is called an AxB cross or an (AxB)F1 or simply an F1.

<sup>41</sup> H. Cosenza and H. Köhler, (1972) Specific suppression of the antibody response by antibodies to receptors. Proc. Nat. Acad. Sci. 69, 2701-2705.

corresponding antigen) is sometimes called suppression. I will call this unresponsiveness to the antigen for that idio type. I will use "idiotype suppression" in a sense that is consistent with the more usual use of the term suppression, namely an active process, that involves one population actively suppressing the activity of another population of lymphocytes.

Eichmann showed that the suppression of an idio type can be induced in mice using antiidiotypic antibodies, as shown in Figure 6-4a. Low doses (100 ng of idio type binding capacity) of an antiidiotypic resulted in an almost complete suppression of the idio type following challenge with antigen 85 days later.<sup>42</sup> This suppression lasted for more than a year without any indication of recovery.<sup>43</sup> The suppressed state could be transferred to a naïve mouse using as few as  $10^5$  spleen cells as shown in Figure 6-4b, but it took 6 weeks following transfer of the cells for the suppression to become complete. He could take similar small numbers of spleen cells from the second mouse and use them to suppress the idio type in a third mouse, and so on for four consecutive transfers spaced at three month intervals. He showed that the cells responsible for the suppression were T cells.

Kölsch and colleagues showed that a suppressed state induced by small doses of antigen in a mouse can likewise be transferred into a naïve mouse using T cells from the suppressed mouse.<sup>44</sup> This was important because it established a direct link between low dose tolerance (induced by antigen) and antigen-specific suppression.

### Allotype-specific suppression

Allotype suppression is a phenomenon that has been studied in two species, namely the rabbit<sup>45,46</sup> and the mouse.<sup>47,48,49</sup> It was discovered in the rabbit in 1962,

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<sup>42</sup> K. Eichmann (1974) Idiotypic suppression. I. Influence of the dose and of the effector functions of anti-idiotypic antibody on the production of an idio type. *Eur. J. Immunol.* 4, 296-230.

<sup>43</sup> K. Eichmann (1975) Idiotypic suppression. II Amplification of a suppressor T cell with antiidiotypic activity. *Eur. J. Immunol.*, 5, 511-517.

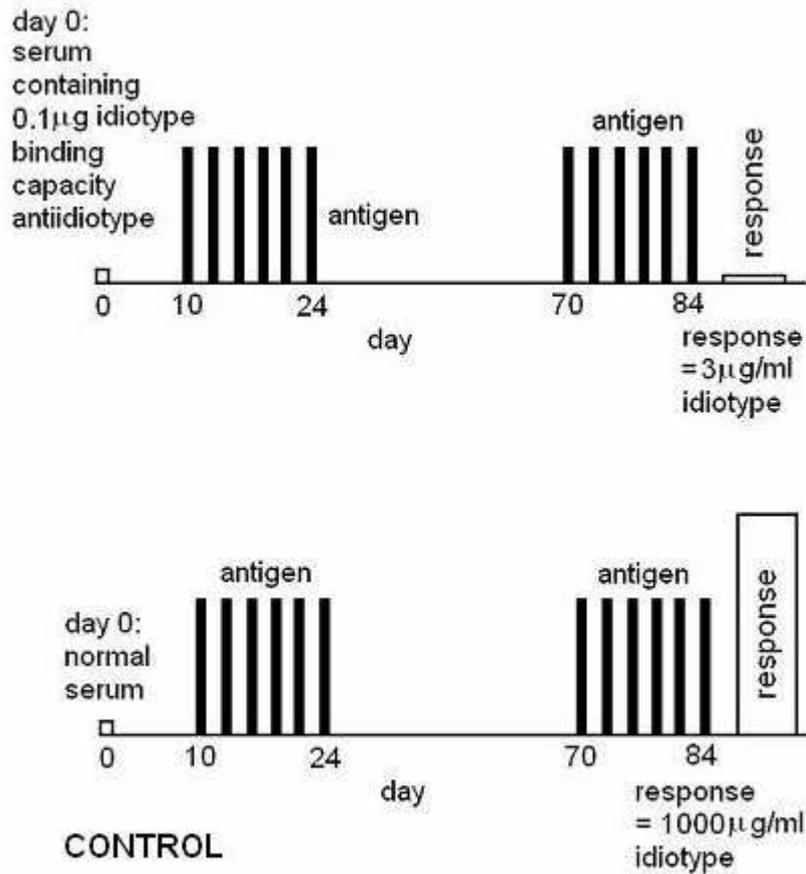
<sup>44</sup> E. Kölsch, R. Stumpf and G. Weber (1975) Low zone tolerance and suppressor T cells. *Transpl. Rev.* 26, 56-86.

<sup>45</sup> S. Dray (1962) Effect of maternal isoantibodies on the quantitative expression of two allelic genes controlling  $\gamma$ -globulin allotypic specificities. *Nature (London)* 195, 677-680.

<sup>46</sup> R. G. Mage and S. Dray (1965) Persistent altered phenotypic expression of allelic  $\gamma$ G-immunoglobulin allotypes in heterozygous rabbits exposed to isoantibodies in fetal and neonatal life. *J. Immunol.* 95, 525-535.

<sup>47</sup> E. B. Jacobson and L. A. Herzenberg (1972): Active suppression of immunoglobulin allotype synthesis. I. Chronic suppression after perinatal exposure to maternal antibody to paternal allotype in (SJLxBALB/c)F<sub>1</sub> mice. *J. Exp. Med.* 135, 1151-1162.

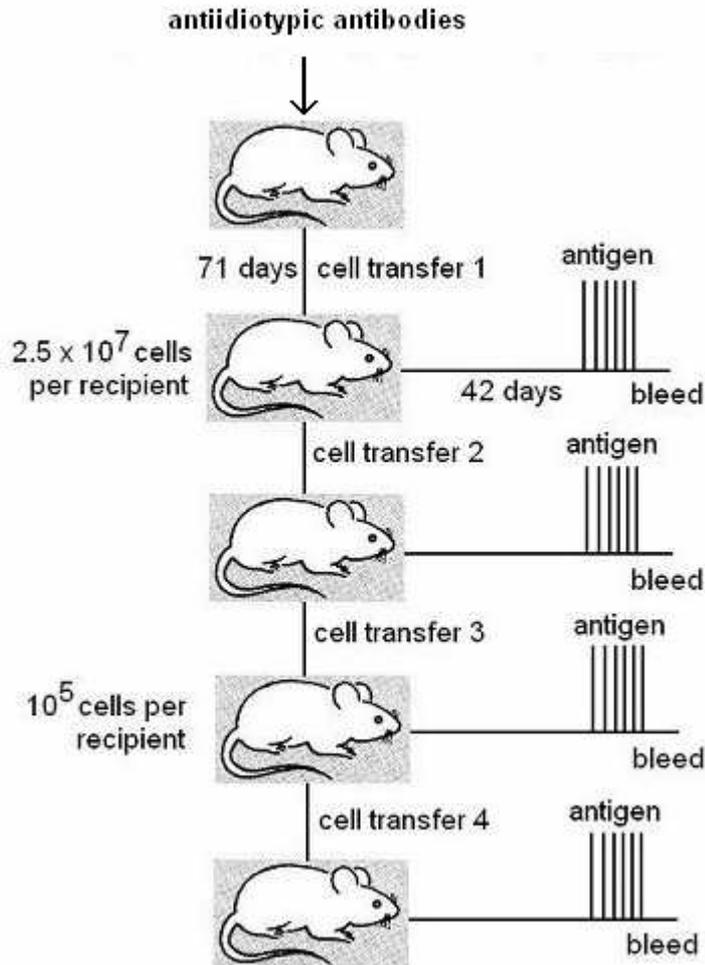
Figure 6-4a Suppression of the component of an immune response bearing a particular idiotype in an experiment by Eichmann. In response to the antigen Group A streptococci, strain A/J mice characteristically make antibodies with an idiotype called A5A. This idiotype can be detected using a serum containing anti-A5A (antiidiotypic) antibodies. Injection of naïve mice with low doses of the antiidiotypic antibodies induce the suppressed state for that idiotype in naïve mice.



<sup>48</sup> E. B. Jacobson, L. A. Herzenberg, R. Riblit and L. A. Herzenberg (1972) Active suppression of immunoglobulin allotype synthesis. II. Transfer of suppressing factor with spleen cells. *J. Exp. Med.* 135, 1163-1176, 1972

<sup>49</sup> Herzenberg, L. A., Chan, E. L., Ravitch, M.M., Riblet, R., J., and L. A. Herzenberg, L. A., (1973) Active suppression of immunoglobulin allotype synthesis. III. Identification of T cells as responsible for suppression by cells from spleen, thymus, lymph node and bone marrow. *J. Exp. Med.* 137, 1311-1324.

Figure 6-4b. Mice are injected with serum containing antiidiotypic antibodies. After 71 days the suppressed state for the A5A idiotype can be transferred to other naïve mice by injecting them with as few as  $10^5$  spleen cells from suppressed mice. An interval of six weeks after transfer of the cells is needed for the suppression to become complete. Then the newly suppressed mouse can similarly serve as the source of suppressor T cells in a further generation of suppressed mice, and so on for at least four consecutive transfers at three month intervals. Hence there must be a mechanism for autocatalytic generation of the suppressed state, starting with small numbers of idiotype-specific suppressor T cells. This is discussed in the context of the symmetrical network theory in chapter 10.



before the discovery of suppressor T cells, and was later shown to involve suppressor T cells in the mouse system. Allotype suppression is illustrated in Figure 6-5. Consider two strains A and B that make IgG antibodies of two different allotypes; let us call them allotype a and allotype b. A genetic cross between these two strains, an AxB animal, typically makes IgG antibodies that are about 50% of the a allotype and about 50% of the b allotype.<sup>50</sup> If however, prior to becoming pregnant, the A strain mother is injected with anti-(b allotype) antibodies, the AxB off-spring can be completely suppressed for the production of IgG antibodies of the b allotype. This phenomenon is not seen with all strains, but when it is seen, it is a dramatic effect. The AxB animal is then suppressed for the production of fully half of the antibodies the animal is genetically capable of making! It can be shown that this is a suppressor T cell phenomenon in experiments in which the transfer of T cells from the treated AxB animal into a normal AxB recipient specifically suppresses the production of the b allotype also in that recipient.<sup>48,49</sup>

An important aspect of allotype suppression is that the B cells that can potentially make the suppressed allotype are present, but they are not actively making and secreting antibodies. Hence the suppression is not simply a case of the cells being deleted and removed from the system; the cells are instead being actively prevented from secreting antibodies.

### Isotype-specific suppression

Treatment of spleen cells with antibodies to the constant part of the cell surface receptors (anti-isotype antibodies) can induce proliferation of the treated cells. On the other hand, injection of these antibodies into a mouse at the time of injection with an antigen suppresses the immune response of the corresponding isotype. Anti- $\gamma$  and anti- $\alpha$  antibodies (anti-isotype) suppress only IgG and IgA production respectively, while anti- $\mu$  suppresses all classes<sup>51</sup>. This difference is ascribed to the fact that cells express and secrete IgM before they switch to the production of IgA and IgG, so the entire developmental pathway can be blocked by anti- $\mu$ , but not by anti- $\alpha$  or anti- $\gamma$ .

### Specific suppressor T cells in self tolerance

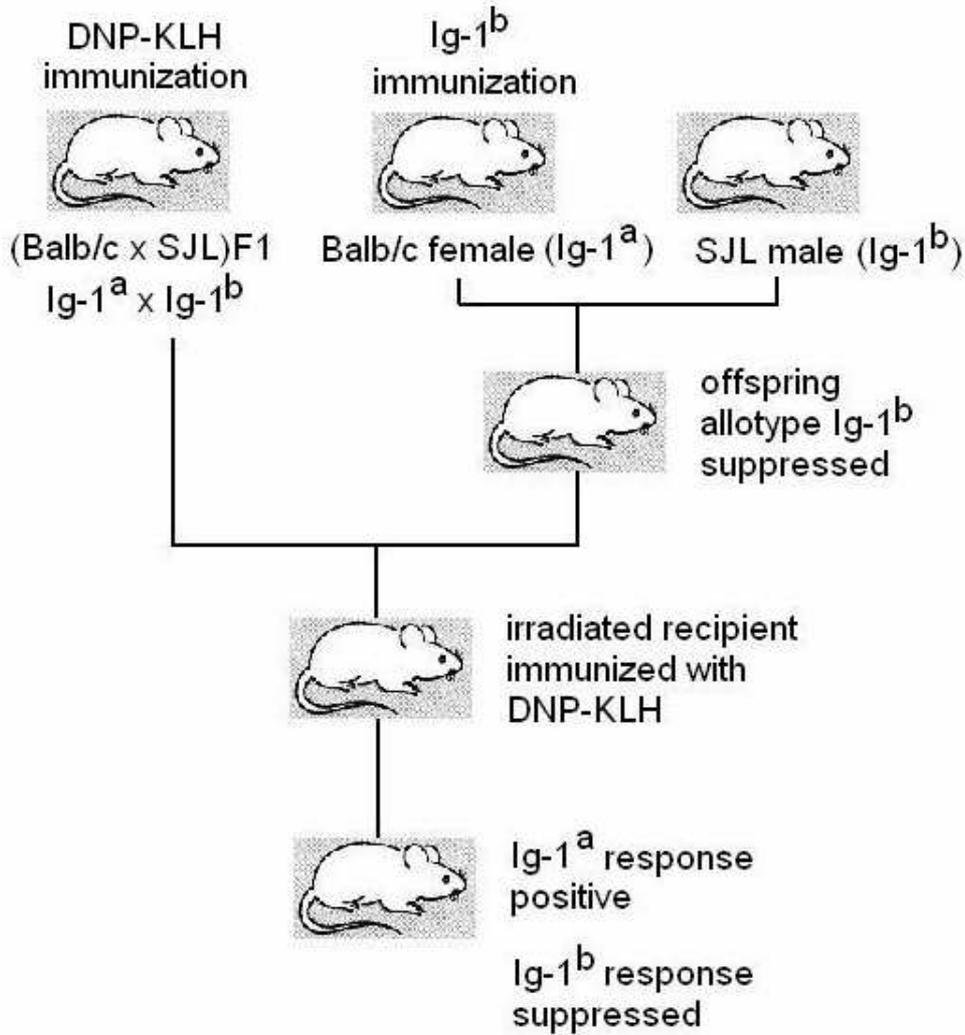
Usually we do not make immune responses to self antigens, but injection with a foreign antigen that resembles a self antigen can break tolerance for the self antigen.

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<sup>50</sup>The allotypes in non-suppressed rabbits can have ratios that are distinctly different from 50:50. For example the ratio of b4 to b9 allotype in b4/b9 heterozygous rabbits is 80:20.

<sup>51</sup>M. D. Cooper, J. F. Kearney, W. E. Gathings, and A. R. Lawton (1980) Effects of anti-Ig antibodies on the development and differentiation of B cells. *Immunol. Rev.* 52, 29-53.

Figure 6-5 The phenomenon of allotype suppression. Balb/c mice and SJL mice have the IgG allotypes Ig-1<sup>a</sup> and Ig-1<sup>b</sup> respectively. A Balb/c female is immunized with Ig-1<sup>b</sup> antibodies so that it makes an anti-Ig-1<sup>b</sup> response. It is then mated with an SJL male. A significant fraction of the Balb/c x SJL offspring do not make any Ig-1<sup>b</sup> antibodies. This is an actively suppressed state, since combining cells from a DNP-KLH (hapten-carrier) immunized mouse with T cells from an allotype suppressed mouse in an irradiated recipient, that is also immunized with DNP-KLH, results in the production of Ig-1<sup>a</sup> anti-DNP antibodies but no corresponding Ig-1<sup>b</sup> antibodies.



An example of such a breakdown of the immune system in discriminating between self and nonself was provided by experiments by Playfair and Marshall-Clarke in 1973, who reported that mice injected with red blood cells from rats made antibodies that are specific not only for rat red blood cells, but also for mouse red blood cells.<sup>52</sup> This shows clearly that the mice have lymphocytes that are specific for self antigens, but that they are somehow normally held in check. Adoptive transfer experiments showed that autoantibody production is in fact regulated by suppressor T cells.<sup>53,54</sup> The suppressor cells apparently regulate autoantibody specifically, in the sense that with time they are able to distinguish between lymphocytes specific for rat red blood cells and lymphocytes specific for mouse red blood cells. In an experiment in which mice were immunized with rat red blood cells each week for 69 weeks, the number of mice producing autoantibodies (antibodies against self) diminished significantly from about the 35<sup>th</sup> week, while the serum level of antibodies to the foreign rat red blood cells persisted.

### **In vitro cellular immunology experiments**

The experiments of Figures 6-1, 6-2 and 6-3 all involve using the mouse as a living test tube. An important advance in analyzing the system was the demonstration in 1967 by Mishell and Dutton that immune responses can be obtained using lymphoid cells in petri dishes, proving that the rest of the mouse is not required for the production of antibodies.<sup>55</sup> Such *in vitro* experiments have been important for quantifying various aspects of the system, for example determining what fraction of B cells are able to make antibodies that bind to a particular antigen, and for providing information concerning the mechanisms by which T cells help and suppress B cell responses.

An *in vitro* immune response can be obtained by removing the spleen of, for example, a mouse, breaking up the spleen into single cells (making a "single cell suspension"), and putting the cells into culture with appropriate nutrients and an antigen. The spleen cells include B cells, T cells and other non-specific accessory cells that are all needed for making an immune response. After a few days (typically 5 to 7

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<sup>52</sup> J. H. L. Playfair and S. Marshall-Clarke (1973) Induction of red cell autoantibodies in normal mice. *Nature New Biology*, 243, 213-214.

<sup>53</sup> J. D. Naysmith, C. J. Elson, M. Dallman, E. Fletcher and M. G. Ortega-Pierres (1980) Anti-erythrocyte autoantibody production in mice associated with the injection of rat erythrocytes. I. Regulation of the response by suppressor cells. *Immunology* 39, 469-479.

<sup>54</sup> K. O. Cox and A. Howles (1981) Induction and regulation of autoimmune hemolytic anemia in mice. *Immunol. Rev.* 55, 31-53.

<sup>55</sup> R. I. Mishell and R. W. Dutton (1967) Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* 126, 423-442.

days) antibody production by the antigen specific B cells can be detected by any one of a variety of assays.

### **Antigen-specific and nonspecific T cell factors**

One of the results from *in vitro* experiments has been that T cells can exert their helping and suppressing effects on B cells via protein molecules called T cell factors, of which some are specific for the antigen ("antigen-specific T cell factors"), and others are not specific for antigen ("non-specific T cell factors"). Antigen-specificity must be mediated by V regions, in order that the great diversity of antigens is matched by a corresponding diversity of recognizing structures. Hence not only antibodies but also antigen-specific T cell factors must have V regions. Antigen-specific T cell factors are thus of more interest for the development of immune network theory than antigen non-specific factors. Non-specific factors help or suppress all clones equally, regardless of their specificity.

As the name implies, an antigen-specific T cell factor binds specifically to a corresponding antigen. Antigen-specific T cell factors are however clearly different from antibodies. For example, they have a molecular weight of approximately 50,000 daltons, which is much less than the molecular weight of the smallest antibody molecules (IgG, with a molecular weight of approximately 150,000 daltons). The first report of such factors of which I am aware was a paper by Nelson in 1970.<sup>56</sup> The literature on such factors is extensive<sup>57</sup>.

### **Antigen-specific suppressor T cell factors**

Antigen-specific T cell factors (without cells) can suppress immune responses. For example, Tada, Taniguchi and their collaborators showed that if a mouse is immunized twice with a protein antigen (KLH or HGG in their experiments), cell free supernatants obtained from the lysates of spleen or lymph node cells are able to suppress immune responses to the respective antigens.<sup>58,59</sup> The suppression is

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<sup>56</sup> D. S. Nelson (1970) Studies on cytophilic antibodies. A mouse serum "antibody" having an affinity for macrophages and fast  $\chi$ -globulin mobility. *Austral. J. Exp. Biol. Med. Sci.* 48, 329-341.

<sup>57</sup> For a table summarizing some of the early results on specific T cell factors, see G. W. Hoffmann (1978) Incorporation of a Non-specific T Cell Dependent Helper Factor into a Network Theory of the Regulation of the Immune Response, in "Theoretical Immunology", G.I. Bell, A.S. Perelson and G.H. Pimbley (eds.) Marcel Dekker, N.Y., 571-602.

<sup>58</sup> T. Takemori and T. Tada (1975) Properties of antigen-specific suppressive T cell factor in the regulation of antibody response of the mouse. I. In vivo activity and immunochemical characterization. *J. Exp. Med.* 142, 1241-1253.

specific, in the sense that factors obtained using KLH suppress immune responses to KLH but not HGG, and factors obtained using HGG suppressed an HGG response but not a KLH response (criss-cross specificity, Figure 6-6). Specific suppression was demonstrated in both *in vitro* and *in vivo* versions of these experiments.

### Antigen-specific helper T cell factors

T cells are also found to be able to mediate help to B cells via soluble secreted factors.<sup>60,61,62</sup> Some helper factors are non-specific; they help B cells non-discriminately, while others are antigen-specific. The amount of specific help mediated by antigen-specific helper factors is typically less striking than the suppression that can be caused by antigen-specific suppressor factors. Antigen specific T suppressor factors have been more extensively characterized than antigen specific T helper factors, and are thus more firmly entrenched as a phenomenon in the cellular immunology literature.

### Contrasuppressor T cells

Another class of T cells involved in regulation are contrasuppressor cells, which, as their name implies, counteract the effect of suppressor cells.<sup>63</sup> An experiment demonstrating the contrasuppression phenomenon is shown in Figure 6-7. In this experiment Ly-1 is a marker of helper T cells and Ly-2 (later renamed CD8) is a marker of both suppressor T cells and contrasuppressor T cells. The *in vitro* response of a million Ly-2 depleted spleen cells (containing B cells and helper T cells) to the antigen sheep red blood cells is markedly suppressed by just  $3 \times 10^4$  Ly-2 cells that had been primed for four days *in vitro* with the antigen. The effect of the Ly-2

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<sup>59</sup> M. Taniguchi, M., Hayakawa, K., and T. Tada (1976) Properties of antigen-specific suppressive T cell factor in the regulation of antibody response of the mouse. II. *In vitro* activity and evidence for the I region gene product. *J. Immunol.* 116, 542-548;

<sup>60</sup> S. Howie and M. Feldmann (1977) *In vitro* studies on H-2-linked unresponsiveness to synthetic polypeptides. III. Production of an antigen-specific T helper cell factor to (T,G)-A--L. *Eur. J. Immunol.*, 7, 417-421.

<sup>61</sup> C. Shiozawa, M. B. Longenecker and E. Diener (1980) *In vitro* cooperation of an antigen-specific T cell-derived helper factor, B cells, and adherent cells or their secretory product in a primary IgM response to chicken MHC molecules. *J. Immunol.* 125, 68-73.

<sup>62</sup> C. Shiozawa, S. Sawada, M. Inazawa and E. Diener (1984) Triggering of affinity-enriched B cells. II. Composition and B cell triggering properties of affinity-purified, antigen-specific, T cell-derived helper factor. *J. Immunol.* 132, 1892-1899.

<sup>63</sup> R. K. Gershon, D. D. Eardley, S. Durum, D. R. Green, F. W. Shen, K. Yamauchi, H. Cantor and D. B. Murphy (1981) Contrasuppression: A novel immunoregulatory activity. *J. Exp. Med.* 153: 1533-1546.

Figure 6-6. Antigen-specific suppression by antigen specific T cell factors. The carrier proteins in this experiment are KLH (keyhole limpet hemocyanin) and BGG (bovine gamma globulin), and the immune response measured is to the hapten DNP (dinitrophenyl) coupled to each of the carriers. Cells from the thymuses and spleens of KLH or BGG primed mice were sonicated (broken up by intense sound waves), and cell-free extracts then obtained from the supernatant following ultracentrifugation. The extract was injected into syngeneic mice that were concomitantly immunized with one of the hapten-carrier conjugates together with an adjuvant. The figure schematically shows the results obtained for cells making IgG antibodies to DNP. This experiment by Takemori and Tada included criss-cross results for both the thymus cell extracts (shown) and for spleen cell extracts. In all cases the IgG response for the groups immunized by the antigen, for which the suppressor cells had been primed, was significantly suppressed ( $P < 0.01$ ) compared with the control response.

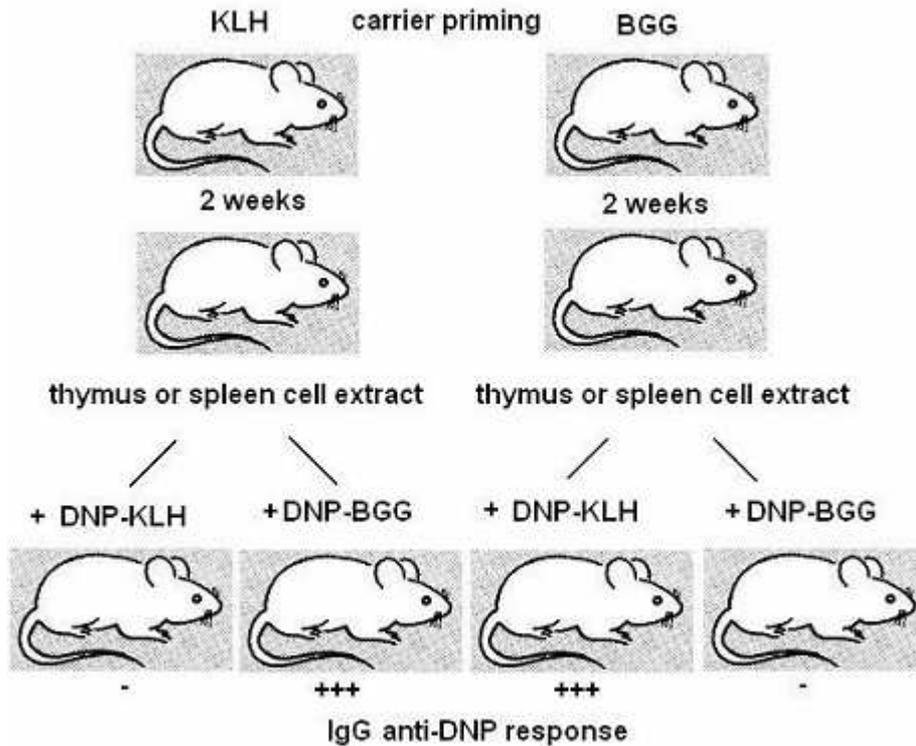


Figure 6-7. The contrasuppression phenomenon. A total of a million B cells and Ly-1 (helper) T cells respond to the antigen, sheep red blood cells, in vitro. This response can be suppressed by just 3% as many Ly-2 (suppressor) T cells, that have been primed by cultivation with the antigen for 4 days. The effect of the suppressors can be fully counteracted by the addition of  $2 \times 10^5$  unprimed T cells, which are therefore labelled contrasuppressors.

Cells			Response
B + Ly-1 T	Ly-2 T primed in vitro with antigen	unprimed T cells	
 $10^6$			 response with B cells and helper T cells
 $10^6$	 $3 \times 10^4$		 antigen-primed Ly-2 T cells suppress response
 $10^6$	 $3 \times 10^4$	 $2 \times 10^5$	 unprimed T cells interfere with suppression

primed suppressor population is cancelled by the addition of  $2 \times 10^5$  normal (unprimed) T cells, with the interpretation that the latter contain contrasuppressor cells. Evidence that the contrasuppressors are distinct from helper and suppressor cells includes the finding that the contrasuppressors express both Ly-1 and Ly-2.

### Hypogammaglobulinemia: suppression in humans

Hypogammaglobulinemia is a human medical condition characterized by abnormally low amounts of gamma globulin, that is, IgG. Patients with this condition have T cells that suppress B cell maturation and antibody production.<sup>64</sup> Here the T cells,

<sup>64</sup> T. A. Waldmann, S. Broder, R. M. Blaese, M. Durm, M. Blackman and W. Stober (1974) Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinemia *Lancet* ii, 609-613.

rather than suppressing one idiootype or one of two allotypes, non-specifically suppress all IgG secreting cells.

### **A lull in the level of interest in suppression**

Extensive studies, including genetic studies, have confirmed that suppression and antigen-specific T cell factors are real phenomena. It became unfashionable however for researchers to work on suppression or antigen-specific factors during the early 1980s because of a paradox called the I-J paradox, which we will discuss in detail in chapter 13. Briefly, suppressor factors express an antigenic determinant called I-J, which is detectable with anti-I-J antibodies. The paradox lay in a conflict between results obtained using classical, serological mapping of the presumed I-J gene and mapping using molecular genetics. Rather than focusing on the paradox as they should have done, and determining whether the experimental data were sound, immunologists deserted I-J and anything related to it, including suppressor T cells and network theory. Only later was it shown that the I-J paradox can in fact be understood in the context of network theory. But by that time most immunologists had lost interest in I-J. That interest needs to be rekindled.