# Self-Nonself Discrimination by T Cells

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**The αβ T cell receptor (TCR) recognizes antigens that are presented by major histocompatibility complex (MHC)–encoded cell surface molecules by binding to both the antigen and the MHC molecules. Discrimination of self from nonself antigens and MHC molecules is achieved by negative and positive selection of T cells in the thymus: potentially harmful T cells with receptors that bind to self antigens plus self MHC molecules are deleted before they can mount immune responses. In contrast, the maturation of useful T cells with receptors that bind foreign antigens plus self MHC molecules requires the binding of their receptor to MHC molecules on thymic epithelium in the absence of foreign antigen. The binding of the TCR to either class I or class II MHC molecules directs differentiation of the selected cells into either $CD4^-8^+$ (killer) or $CD4^+8^-$ (helper) T cells, respectively.**

THE IMMUNE SYSTEM DISTINGUISHES ANTIGENS BY DIverse, clonally distributed antigen receptors that are expressed on the surface of B and T lymphocytes (*1*). The receptor on B lymphocytes is immunoglobulin, which can be secreted after antigenic stimulation. Immunoglobulins, also called antibodies, can bind to a large variety of soluble as well as cell-bound antigens. The T cell receptor (TCR) is not secreted and exists in two heterodimeric forms either consisting of α and β (αβ TCR) (*2*) or γ and δ (γδ TCR) (*3*) proteins. The antigens recognized by the γδ TCR are still being defined, whereas it has been concluded that the αβ TCR binds to protein fragments presented by class I or class II major histocompatibility complex (MHC)–encoded molecules (*4*). It appears that the αβ TCR binds to both the peptide as well as polymorphic residues of MHC molecules. Antigen recognition by T cells is therefore called MHC-restricted. The antigen recognition of cell-bound antigens by the αβ TCR is facilitated by CD4 and CD8 coreceptors expressed in a mutually exclusive fashion on mature T cells (*5*). CD4 and CD8 coreceptors bind to nonpolymorphic residues of class II and class I MHC molecules, respectively (*6*).

Antigen receptors of the immune system are generated throughout life by random rearrangement of DNA segments (*7*) encoding the variable parts of the receptors and by the mostly random pairing of variable protein chains. This means that receptors with specificity for nonself as well as for self antigens are generated. Thus, the discrimination by the immune system of self and nonself cannot be acquired genetically but must but learned somatically.

Early experiments in hematopoietic chimeras and thymus transplanted mice (*8*) were concerned with learning of the self-nonself discrimination by T cells. In these experiments, the fate of lymphocytes developing in a MHC incompatible environment was studied. It was shown that tolerance to foreign MHC antigens could be acquired by developing T cells. The experiments also suggested that mature T cells preferentially respond to foreign antigens that are presented by those MHC molecules that the immature T cells had encountered during their development in the thymus. The ambiguity in the interpretation of these data was because the readout of these studies employed lymphocyte reactivity in functional assays rather than the analysis of specific receptors on resting lymphocytes. Such studies, therefore, could never resolve whether lymphocytes bearing certain receptors were silent or absent. In retrospect, it is understandable that these experiments permitted the advancement of almost any hypothesis regarding self-nonself discrimination. In particular, they left the question open whether or not tolerance could result from deletion of autoaggressive lymphocytes. They failed to provide an answer to the question of whether binding of TCRs to thymic MHC antigens in the absence of foreign antigen was an essential step in T cell maturation, resulting in positive selection of T cells whose receptors are able to recognize foreign antigens presented by self MHC molecules. Finally, they did not address the question of which factors determined the CD4/CD8 phenotype of mature T cells (*9*).

A conclusive study of intrathymic selection events requires that one can follow the fate of individual lymphocytes bearing certain antigen receptors throughout the development of the immune system. Until recently this was impossible, because receptors of defined specificity, exhibiting markers recognized by monoclonal antibodies, were too infrequent to be unambiguously identified among other receptors of seemingly endless diversity. Recent approaches, however, permitted the identification of lymphocytes bearing certain receptors during T cell development and have exposed hypotheses (*9*) to direct experimental testing.

One approach was based on the analysis of diverse T cell receptors that share sequences in the TCRβ chain that can be detected by monoclonal antibodies (*10–14*). The limitation of this approach with regard to uncovering the principles of self-nonself discrimination is the heterogeneity and multispecificity of these receptors. It is not surprising, therefore, that experiments in these systems did not provide conclusive answers to the questions of whether or not the binding of T cell receptors to thymic MHC molecules was an essential step in T cell maturation and in which way the CD4/CD8 phenotype of mature T cells was determined.

Another approach consisted of artificially increasing the frequency of cells with one receptor specific for a conventional antigen presented by MHC antigens. This was achieved in TCR transgenic mice that contained both β and α TCR transgenes (*15–19*). Because some of these transgenic animals expressed essentially only one receptor, one could envisage drawbacks in studying mice with a monospecific T cell compartment: for instance, one could not

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exclude that diverse receptors were essential for T cell development. Also, the premature or too high expression of TCR transgenes that are already rearranged in the germline could interfere with normal T cell differentiation. Fortunately, none of these appeared to impose a severe problem as T cell development in primary lymphoid organs was not impaired in TCR transgenic mice. It was also possible to compare effects caused by superantigens, which include unknown cell surface molecules (*10*) and bacterial toxins (*11*), as they bound to T cell receptor β chains in normal and TCR transgenic mice, to reveal possible abnormalities in the latter. The great advantage of the transgenic system was that the intrathymic selection of a single αβ TCR with defined specificity for a conventional antigen and presenting MHC molecule could now be studied. In the following we will summarize results and conclusions that were made possible especially through the use of transgenic models of self-nonself discrimination.

## Negative Selection of Self-Reactive Cells

*Deletion of potentially harmful T cells at an immature developmental stage.* Mature $CD4^+8^-$ helper and $CD4^-8^+$ killer T cells with αβ TCRs develop in the thymus from $CD4^-8^-$ precursors through $CD4^+8^+$ intermediates (*20*). The relationship of $CD4^+8^+$ cells, which constitute the majority of thymocytes, to mature T cells has puzzled immunologists for a long time because most of these cells die intrathymically (*21*). Experiments in TCR transgenic mice have indicated that these cells are indeed intermediates in the developmental pathway of T cells.

TCR transgenic mice were produced that contained the α and β TCR transgenes, which together encode a receptor specific for the male (H-Y) antigen presented by class I H-$2D^b$ molecules. When thymocytes of these animals were analyzed, it was found that most thymocytes expressed both transgenic α and β TCR chains. While the thymus of female mice was of normal size, the male thymus was very small and contained virtually no $CD4^+8^+$ thymocytes (Fig. 1). The absolute number of $CD4^-8^-$ precursors expressing the transgenic receptor was the same in male and female mice, showing that these cells were not the target of deletion. The virtual absence of $CD4^+8^+$ thymocytes in the male mice indicated that thymocytes could be deleted by their ligand as soon as they began to express CD8 coreceptors (*15*), which facilitate recognition of antigens presented by class I MHC molecules.

In some cells that initially bore transgenic α and β TCR chains on the cell surface, endogenous α TCR genes could rearrange (*22*) such that at later stages of development, some thymocytes expressed receptors composed of the transgenic β TCR chain and endogenous α TCR chains. Cells with this phenotype included most $CD4^+8^-$ T cells, which were numerous in female but were greatly reduced in male TCR transgenic mice, despite no longer bearing a male-specific TCR (*15*). Because of this and because only H-Y–specific cells that expressed CD8 were deleted, it was concluded that $CD4^+8^+$ male-specific thymocytes contained the precursors of nonmale-specific $CD4^+8^-$ progeny. These conclusions were reinforced by experiments in normal mice that were concerned with deletion caused by superantigens (*23*).

Collectively, these experiments provided the first direct evidence that $CD4^+8^+$ thymocytes, which do not respond to antigenic stimulation in a conventional manner when compared to mature T cells (*24*), represent the intermediate developmental stage that is sensitive to antigen-induced deletion. Deletion occurs when the αβ TCR binds to the complete ligand, consisting of the specific peptide presented by restricting MHC molecules. Similar conclusions were reached in other transgenic systems (*16, 19*).

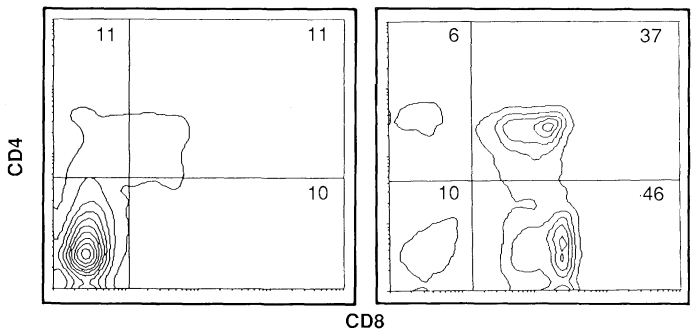


**Fig. 1.** Thymocyte subpopulations defined by CD4 and CD8 surface antigens in male (left) and female (right) *H-2*$^b$ αβ TCR transgenic mice (TG71) expressing a TCR specific for male (H-Y) antigen presented by H-$2D^b$ MHC molecules. The thymus from male mice contained about $10^7$ thymocytes, while the thymus from female contained about $10^8$ thymocytes. Most thymocytes, with the exception of the $CD4^+8^-$ thymocytes, expressed both transgenic TCR chains on the cell surface. Numbers in quadrants refer to percentages of cells.

*Partial and complete deletion of immature thymocytes.* Complete deletion of $CD4^+8^+$ thymocytes by self antigens was observed in TCR transgenic mice, whereas experiments in normal mice were interpreted to indicate that only a fraction of $CD4^+8^+$ thymocytes was susceptible to deletion (*25*). Because different antigens were under study in the experiments conducted in transgenic or normal mice, it was not clear whether the extent of deletion among $CD4^+8^+$ thymocytes was determined by the nature of the different experimental animals (transgenic versus nontransgenic) or by the use of different antigens, (class I MHC–presented peptides versus superantigens) (*15, 19, 25–27*). We therefore compared the deletion induced by class I MHC–presented peptides and the deletion caused by the superantigen staphylococcal enterotoxin B (SEB) in TCR transgenic mice. SEB, which binds to $V_\beta8$ TCR proteins, was reported to delete no more than 50% of $CD4^+8^+$ thymocytes expressing the $V_\beta8.2$ gene segment after neonatal tolerance induction in normal mice (*28*). Because our transgenic mice express $V_\beta8.2$ TCRβ chains, we used the same protocol of neonatal tolerance induction to check for possible abnormalities in transgenic mice. In contrast to male transgenic mice in which all $CD4^+8^+$ $V_\beta8.2$ T cells were deleted, we found that SEB in female αβ TCR transgenic mice deleted less than 50% of $CD4^+8^+$ thymocytes expressing the $V_\beta8.2$ transgene (Fig. 2). The effect of both endogenous self H-Y antigen and exogenous SEB on peripheral lymphoid tissue was indistinguishable in that there were virtually no T cells expressing normal coreceptor levels. These results indicate that superantigens and conventional antigen can delete different proportions of $CD4^+8^+$ thymocytes. A similar difference in the extent of deletion induced by class I MHC–presented peptides and superantigens was also reported by Pirchner and co-workers (*19*). Therefore, the nature of antigen and not the nature of the mice determined the extent of deletion among immature $CD4^+8^+$ thymocytes in these experiments.

The above experiments established that, as postulated by Lederberg (*29*), deletion of immature lymphocytes plays a major role in tolerance to self because deletion is not only induced by antigens expressed in the thymus, but also by antigens that reach the thymus from the periphery, such as SEB given intraperitoneally after birth. This means that soluble proteins present in the body are able to induce deletion of immature cells in the thymus. While these experiments have clarified the mode of tolerance induction in developing T cells, the tolerization of mature T cells by antigens which do not reach developing T cells remains an enigma that is being addressed in various transgenic models.

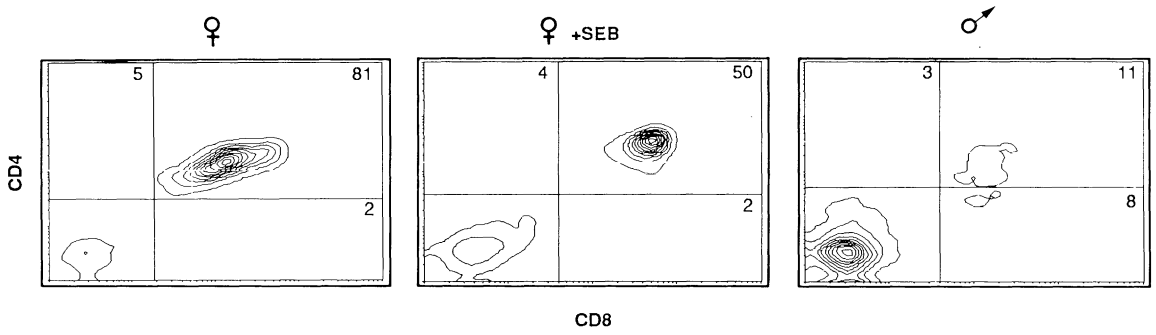


**Fig. 2.** Thymocyte subpopulations defined by CD4 and CD8 surface antigens from 12-day-old αβ TCR transgenic mice expressing a TCR specific for male (H-Y) antigen presented by H-2D$^b$ MHC molecules. In some female αβ TCR transgenic mice, staphylococcal enterotoxin B (SEB) was injected intraperitoneally (20 μg) at 2-day intervals from birth onwards. The vast majority of thymocytes (>90%) expressed both the transgenic β and α TCR chain.

## Positive Selection of Self MHC–Restricted T Cells

*Maturation induced by restricting MHC molecules.* The transgenic model established for the first time that the expression of restricting MHC molecules without the specific peptide on thymic radioresistant tissue was essential for the maturation of CD4$^+$8$^-$ and CD4$^-$8$^+$ thymocytes from immature CD4$^+$8$^+$ precursors (*30–32*). This was especially obvious in TCR transgenic SCID mice that, because of a defect in the rearrangement process, could only express the already rearranged transgenic TCR genes (*32*). In these animals T cell development was arrested at the CD4$^+$8$^+$ intermediate developmental stage unless the restricting MHC molecule was expressed on thymic tissue (Fig. 3) (*32*). In contrast to negative selection (deletion), which prevents the maturation of potentially harmful self-specific T cells, positive selection is a mechanism of "self assertion," which selects useful T cells for entry into the peripheral T cell pool (*33*). Once T cells have matured, they cannot respond to the selecting MHC molecules alone, but they can respond when the self MHC molecule presents a certain foreign peptide. In the case described here, male-specific T cells were selected by the restricting MHC molecules in a female mouse. The selected, mature T cells responded to male but not to female hematopoietic cells that expressed the restricting MHC molecules (*15*).

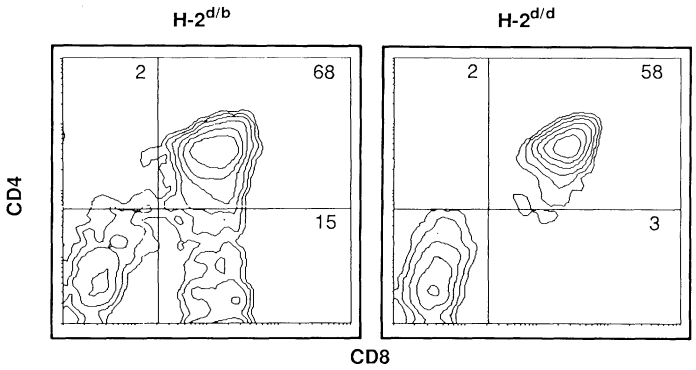


**Fig. 3.** Thymocyte subpopulations defined by CD4 and CD8 surface antigens from female *H-2$^{d/b}$* (left) and *H-2$^d$* (right) αβ TCR transgenic SCID mice. Note the lack of mature thymocytes in animals lacking the presenting H-2D$^b$ MHC molecule and the absence of mature CD4$^+$8$^-$ thymocytes in animals expressing the presenting H-2D$^b$ MHC molecule. Numbers in quadrants refer to percentages of cells.

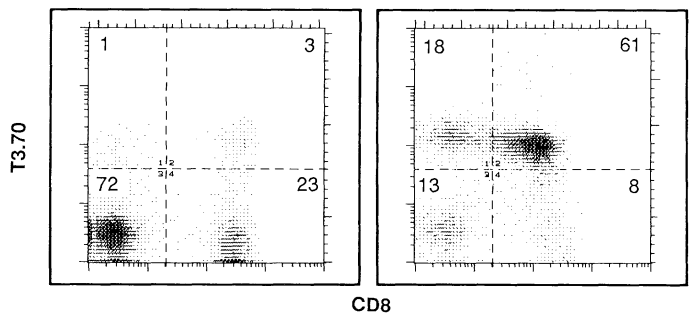


**Fig. 4.** The expression of the transgenic TCRα chain by lymphocytes from lymph nodes of female (left) and male (right) αβ TCR transgenic mice derived from the transgenic strain TCR 57. Most T cells in female mice are CD4$^+$8$^-$, and all of these and most of the CD4$^-$8$^+$ T cells express endogenous α chains paired with transgenic β chain. In the male most T cells express the transgenic α and β chain, and a high proportion of cells expresses CD8 coreceptors at levels one-third to one-half those in females.

*The TCR specificity determines the CD4/CD8 phenotype.* In TCR transgenic mice, the specificity of the TCR for class I or class II MHC molecules determined whether the mature T cell that expressed the particular receptor was of the CD4$^-$8$^+$ (killer) or CD4$^+$8$^-$ (helper) phenotype (Figs. 1 and 3). Binding of the TCR to class I or class II MHC molecules in the thymus in the absence of the specific peptide not only induced maturation, but directed differentiation into the CD4$^-$8$^+$ and CD4$^+$8$^-$ T cells. Thus, in the TCR transgenic mice, a class I MHC–restricted TCR was expressed on only CD4$^-$8$^+$ T cells and resulted in an increased proportion of CD4$^-$8$^+$ thymocytes (*15, 16, 30–32*) (Fig. 1), whereas a class II MHC–restricted TCR was expressed only on CD4$^+$8$^-$ T cells (*17, 18*). This conclusion could not readily be reached from experiments employing superantigens (*10, 12, 13*) but is supported by one particular type of experiment in normal mice in which the positive selection of certain receptors on CD8-positive thymocytes was inhibited by class I MHC antibodies (*14*).

*Positive selection induces differentiation, but not expansion.* The results of experiments in TCR transgenic mice do not support the hypothesis that positive selection represents "skewing" or "bending" of the T cell repertoire, either by the expansion of mature self MHC–restricted T cells (*34*) or by suppression of mature nonself MHC–restricted T cells (*35*). Newly formed CD4$^+$8$^-$ and CD4$^-$8$^+$ mature thymocytes do not divide nor are they the immediate progeny of dividing cells (*36, 37*). Thus thymic MHC molecules do not drive the expansion of mature T cells. In addition, suppression of mature, nonself MHC–restricted T cells likewise cannot explain positive selection, as this cannot occur in mice with a monospecific immune system like TCR transgenic SCID mice (*32*). Therefore, positive selection reflects the differentiation of a resting CD4$^+$8$^+$ thymocyte into a mature CD4$^+$8$^-$ or CD4$^-$8$^+$ thymocyte. This differentiation step is induced by the binding of TCR to thymic class II or class I MHC molecules, respectively.

*Requirement for MHC expression on thymic epithelial cells.* In a variety of systems (*22, 31, 38–40*), including TCR transgenic mice, it has

been established that positive selection does not occur when the restricting MHC molecules are expressed only on hematopoietic tissue of the thymus. This suggested that expression of the relevant MHC antigens by epithelial cells is required for positive selection, even though it was not certain in all of these experiments that the selecting MHC antigens were expressed exclusively on thymic epithelial cells. Expression of MHC antigen on epithelial cells may be essential because these cells provide a special microenvironment, provide additional ligands in addition to the selecting MHC antigens, or both (*9*). In contrast, deletion is easily observed if only hematopoietic tissue carries the restricting MHC molecule plus peptide (*22*). The expression of ligands on epithelial cells only may not cause deletion, but may result in anergy such that the antigen-specific T cells are not absent, but can no longer be stimulated by the antigens (*41*).

## Order of Negative and Positive Selection

Studies in TCR transgenic mice indicate that positive selection can occur at the same or at a later developmental stage than negative selection. It was found that in female αβ TCR transgenic mice there was considerable rearrangement and expression of endogenous α TCR genes during T cell development. It could be shown that the earliest precursor T cells in the thymus expressed both TCR transgenes on the surface (*22*). In spite of this, in female offspring of one particular transgenic founder mouse, it was observed that most mature T cells, including $CD4^+8^-$ and $CD4^-8^+$ T cells, expressed transgenic TCR β chains ($\beta_T$) paired with endogenous TCRα chains ($\alpha_E$) (Fig. 4). This was so because these T cells expressed both endogenous as well as transgenic TCRα genes (*22*) and because some endogenous TCRα chains apparently paired better with the transgenic TCRβ chain than the transgenic TCRα chain such that only the endogenous TCRα chains reached the surface. The $CD4^+8^-$ and $CD4^-8^+$ $\beta_T\alpha_E$ T cells must have undergone positive selection by thymic class II and class I MHC molecules because this is required to generate mature T cells of this phenotype. $CD4^-8^+$ and $CD4^-8^+$ $\beta_T\alpha_E$ T cells were, however, largely absent in male offspring of the same transgenic founder mouse and most T cells expressed $\beta_T\alpha_T$ TCRs but no CD8 coreceptors or 50 to 80% reduced CD8 coreceptor levels (Fig. 4). This was so because in male animals, the deletion eliminated male specific $\beta_T\alpha_T$ $CD4^+8^+$ immature thymocytes before they could express endogenous TCRα genes. Thus, only those T cells that expressed the transgenic TCR, but because of the reduced CD8 coreceptor levels did not react to male cells (*15*), could leave the thymus.

These observations indicate that deletion in male mice eliminated those $\beta_T\alpha_T$ precursor T cells of those $\beta_T\alpha_E$ TCR–expressing cells that in female mice underwent positive selection. Hence, T cells are susceptible to deletion at the same developmental stage that they are susceptible to positive selection or earlier.

## Transgenic Models: Leading or Misleading?

T cell receptor transgenic animals have the advantage that the selection of a single TCR with defined specificity for normal antigen and presenting MHC molecule can be studied. At present, studies in normal mice can only be done by studying the selection of heterogenous receptors. We therefore think that studies in TCR transgenic mice yielded the least ambiguous conclusions with regard to the question of how the immune system learns to distinguish self from nonself. Experiments conducted in nontransgenic mice are quite consistent with observations made in the transgenic models even though on their own they appear less conclusive. Thus, it appears that transgenic models have been quite useful in defining the rules governing T cell repertoire selection (Fig. 5). Many molecular details remain to be worked out—first of all, the nature of MHC ligands inducing positive selection—and it is likely that transgenic mice will significantly aid such studies in the future.

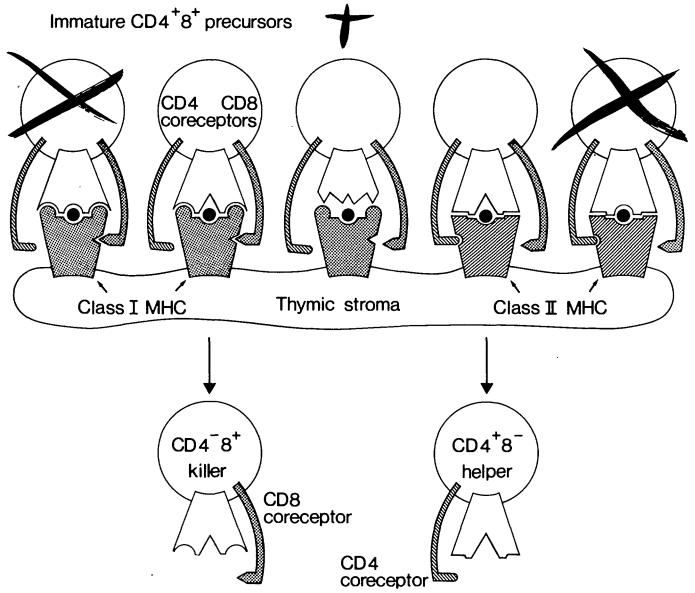


**Fig. 5.** A schematic view of negative and positive selection in the thymus. Class I and class II MHC molecules on thymic stroma present a self peptide (●). Thymocytes with receptors fitting MHC molecule plus self peptide (left or right) are eliminated at an immature stage of T cell development. Thymocytes with receptors fitting MHC molecule but not the peptide (second from left and right) are selected for further maturation. Binding to class I or class II MHC molecule directs differentiation into the $CD4^-8^+$ (killer) and $CD4^+8^-$ (helper) phenotype, respectively. Thymocytes with receptors fitting neither self peptide nor self MHC die from neglect.

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# The Need for Central and Peripheral Tolerance in the B Cell Repertoire

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**The immune system normally avoids producing antibodies that react with autologous ("self") antigens by censoring self-reactive T and B cells. Unlike the T cell repertoire, antibody diversity is generated within the B cell repertoire in two phases; the first occurs by gene rearrangement in primary lymphoid organs, and the second phase involves antigen-driven hypermutation in peripheral lymphoid organs. The possibility that distinct cellular mechanisms may impose self tolerance at these two different phases of B cell diversification may explain recent findings in transgenic mouse models, in which self-reactive B cells appear to be silenced both by functional inactivation and by physical elimination.**

ANTIBODIES HAVE BECOME POWERFUL TOOLS IN RESEARCH and biotechnology. In these areas, as in the acquisition of immunity, production of potentially useful antibodies often depends on two aspects of self-nonself discrimination during the antibody response. First, antibodies are not normally produced against self antigens, and secondly, antibodies to foreign antigens are normally directed exclusively at regions on the foreign antigen that differ from self. The physiological imperative of avoiding autoantibody production has been appreciated since the first demonstration in 1900 of the destructive effects of isoantibodies on genetically mismatched red blood cells (*1*) and the resultant possibility that "formation of autotoxins [autoantibodies] would . . . constitute a danger threatening the organism more frequently and much more severely than all exogenous injuries" (*2*, p. 253). The implications of self tolerance in research and biotechnology are equally profound, since the absence of antibodies directed to self antigens makes it relatively straightforward to generate species-specific antibodies for use in sensitive immunoassays or allele-specific antibodies for blood grouping and tissue typing before transfusion or organ transplantation. Nevertheless, the immunological mechanism responsible for preventing autoantibody production remains controversial.

Historically, self tolerance has long been thought to involve a mirror image of the processes involved in immunity (*3, 4*). Rather than being genetically determined characteristics, both immunity and tolerance were found to be acquired during development of the immune system (*3, 5*). Not surprisingly, the concept that immunity was acquired by "clonal selection" of foreign antigen–specific precursor cells and their differentiation into antibody-secreting cells (*4, 6, 7*) led to the hypothesis that tolerance, as a mirror image, would be acquired by "clonal deletion" or functional inactivation ("clonal anergy") of precursor B lymphocytes that expressed antibodies to self antigens (*4, 8, 9*).

The discovery of T and B lymphocytes (*10*) has necessitated a reappraisal of the validity of this simple model for preventing production of autoantibodies. Since antigen-specific B cells need to collaborate with antigen-specific T cells to mount efficient antibody responses to foreign antigens (*10, 11*), the failure to produce antibodies to self antigens could merely reflect clonal deletion of self antigen–specific T cells rather than any change in the B cells themselves (*12*). However, T cells and B cells collaborate in such a way (*13*) that foreign antigen–specific T cells may, in fact, interact with self-reactive B cells whenever a foreign antigen becomes noncovalently associated with a self antigen, as in the interaction between a viral DNA-binding protein and self DNA (Fig. 1A) or when a foreign antigen happens to cross-react with a self antigen, for example, during the production of species-specific or allele-specific antisera (Fig. 1B). The absence of high-affinity autoantibodies to self antigens in these situations (*14*) therefore indicates that during acquisition of humoral immunity self tolerance not only involves T cell unresponsiveness but also processes that act directly on the B cells. In this article, we focus on the issue of tolerance within the B cell repertoire, with particular emphasis on the similarities and differences to T cell tolerance and on recent work in transgenic mice.

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