The Role of the T Cell Receptor in Positive and Negative Selection of Developing T Cells

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Although many combinations of αβ T cell receptors are available to the T cells in any given organism, far fewer are actually used by mature T cells. The combinations used are limited by two selective processes, positive selection of T cells bearing receptors that will be useful to the host, and clonal elimination or inactivation of T cells bearing receptors that will be damaging to the host. The ways in which these two apparently contradictory processes occur, and the hypotheses that have been suggested to reconcile them, are discussed.

ALTHOUGH A MORE COMPLETE UNDERSTANDING OF MAJOR histocompatibility complex (MHC)–restricted antigen recognition, the hallmark of the T cells that express αβ receptors, awaits the x-ray crystallographic structure of the αβ T cell receptor (TCR) bound to antigen MHC, it has now been explained in general molecular terms (Fig. 1). An antigenic peptide, the end product of complex intracellular biochemical degradation known as "antigen processing," is bound to MHC (class I and, by analogy, class II) in a groove formed by a β-pleated sheet and two α helices (*1*). The variable regions of the α and β chains of the T cell receptor form a combining site that has contact residues for both the bound peptide and the MHC molecule (*2*). The TCR noncovalently associates with a molecular complex, CD3, that is necessary for the membrane expression of the αβ heterodimer, serves to transmit the signal generated by TCR engagement at the cell surface to the interior of the cell, and thereby induces the effector function appropriate to the type of T cell involved (*3*). The accessory molecules, CD4 and CD8, bind monomorphic determinants on the class II and class I MHC molecules, respectively, thus serving to increase the "avidity" of the T cell for its target, and also play a role in T cell signaling (*4*).

The specificity of individual T cells is determined during their development in the thymus. When they arrive from the bone marrow, thymic progenitor cells express no TCR. Rearrangement of variable (V), diversity (D), and joining (J) gene segments at the β locus allows for cytoplasmic expression of the β chain. This event is followed by V-J rearrangement at the α locus, culminating in αβ TCR expression on the cell surface of immature thymocytes beginning at about day 17 of gestation (*5*). (αβ TCR expression is preceded by expression of another TCR, the γδ heterodimer, at day 14 to 15. γδ T cells comprise a separate T cell lineage whose recognition properties and function are only now being elucidated and will not be discussed further in this review.)

Immature αβ thymocytes express an amount of TCR one-fifth to one-tenth that of peripheral T cells (*6*) and, because they coexpress the accessory proteins CD4 and CD8 (*7*), are referred to as "double-positive" thymocytes. Subsequent thymic maturation leads to the mature, "single-positive" stage of development, at which time the T cell expresses only one of the accessory molecules and has increased its density of TCR expression to that of peripheral T cells, approximately 20,000 to 40,000 molecules per cell. A diagram illustrating a likely time course of these and other events that take place during thymocyte maturation (discussed below) is shown in Fig. 2.

Random rearrangement of the TCR gene segments at the $\alpha(V_\alpha J_\alpha)$ and $\beta(V_\beta D_\beta J_\beta)$ loci, the contribution of N region diversity created by imprecise joining of the gene segments or nucleotide insertion at the joining sites, and combinatorial association of the two chains could give rise to a large T cell repertoire of at least 10^9 different specificities, the germline or "unselected" repertoire (*8*). This large repertoire is not expressed in any animal, however, because it is subject to two selection events that occur in the thymus. Positive selection allows the maturation of thymocytes bearing receptors that bind self MHC in the thymus (*9*), whereas negative selection arrests the development of thymocytes bearing self-reactive receptors [reviewed in (*10*)]. The unresolved paradox in the requirement for selection on the basis of self recognition (self MHC) and the restriction against self recognition by the same receptor will be discussed in detail below.

T Cell Specificity, V_β's and Superantigens

Despite the fact that the TCR combining site is shaped by contributions from both the α and β chains, in the case of certain "superantigens," recognition is dominated by V_β alone. Superantigens stimulate most T cells bearing a given V_β, corresponding to a significant percentage of total T cells, whereas conventional antigens, that is, peptides associated with MHC, stimulate only a fraction of a percentage of total T cells. Superantigens can be grouped into two general categories, those expressed endogenously in the organism [self superantigens (*11–14*)] and those that are exogenously derived, for example, the staphylococcal enterotoxins (*15*). The characteristics of superantigens and the existence of monoclonal antibodies (MAbs) directed against specific V_β chains

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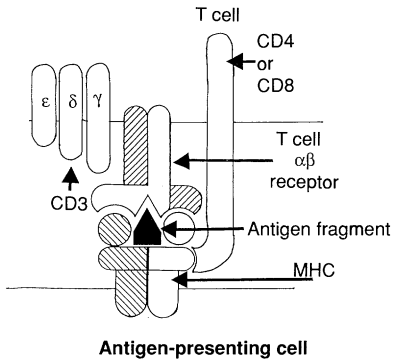

Fig. 1. Diagrammatic structure of the αβ TCR complexed to antigen plus MHC. The T cell αβ receptor has a combining site for antigen-MHC. The antigenic peptide (represented by the black pentagon) is bound by an MHC molecule on the surface of an antigen-presenting cell in a groove created by two α helices (circles) and a β-pleated sheet (platform). Other molecules involved in T cell recognition and activation, the γ, δ, and ϵ chains of CD3, and the "accessory" or "costimulatory" molecules, CD4 and CD8, are shown.

have allowed immunologists to follow the fate of a population of T cells with known specificity on the basis of structural characteristics (that is, V_β expression), rather than reactivity. For example, in the first case described, V_β17a-bearing T cells were found to recognize frequently the class II MHC molecule, I-E (*11*), plus a B cell–derived product (*16*), regardless of any identifiable contribution from other receptor components (*11, 17*). Anti-V_β MAb's have therefore been unexpectedly powerful experimental tools for studies on the T cell repertoire. They offer the advantages of anti-idiotypic antibodies, that is, they bind TCRs with known antigen specificities, and yet they are more useful than anti-idiotypic antibodies because they recognize a large and measurable percentage of the total peripheral T cells in unmanipulated mice.

Positive Selection

Soon after the discovery of MHC-restricted T cell recognition (*18*), it was found that not only do T cells recognize antigen in association with MHC, but almost exclusively in association with self MHC (*9*). This is not an inherent property of the developing T cells but is "learned" during thymocyte maturation. Classic experiments with chimeric mice showed that bone marrow cells injected into irradiated allogeneic or semiallogeneic hosts developed into T cells capable of responding to antigen presented by the MHC of the irradiated host, rather than that expressed by the bone marrow cells themselves. Analysis of chimeras grafted with thymuses depleted of bone marrow–derived cells showed that it was the radioresistant thymic epithelium (that was not derived from bone marrow cells) that imprinted MHC restriction on developing thymocytes and did not necessarily lead to tolerance (*9, 19*). Thus, "self" refers to the *MHC* haplotype expressed on the thymic epithelium in which the thymocytes matured. This process of thymic learning is called positive selection to indicate that thymocytes bearing receptors capable of interaction with self MHC on the thymic epithelium receive a positive signal that triggers their developmental progression to mature thymocytes. However, until recently positive selection could only be measured by the peripheral reactivity of T cells that had developed in chimeric mice and the analysis was subject to possible artifacts generated by complex cellular interactions between allogeneic and semi-allogeneic cells. A more direct demonstration of positive selection awaited the advent of new technology.

First, the requirement for direct interaction between the TCR and MHC during positive selection was demonstrated. Antibodies to TCR or MHC were injected into mice from birth or were added to fetal thymic organ cultures and tested for their effect on thymocyte development (*20*). The MAb treatment interfered with the development of mature cells of the corresponding TCR or MHC specificity, when measured directly by TCR or accessory molecule expression on the mature thymocytes or by function (*20*).

Second, positive selection has been directly, and dramatically, demonstrated in TCR αβ transgenic mice constructed to express a single TCR on all or almost all thymocytes and peripheral T cells (*21, 22*). In these mice, immature thymocytes expressing the transgenic receptor developed regardless of the *MHC* haplotype of the transgenic animals. However, large numbers of mature thymocytes and peripheral cells only appeared in transgenic mice expressing the same *MHC* haplotype as the mouse from which the clone expressing the transgenic receptor was originally isolated. In addition, the CD4:CD8 ratio of the resulting repertoire was strongly skewed in favor of the accessory molecule expressed in the original T cell clone. Thus, positive selection was shown to involve three molecules: TCR, MHC, and CD4 or CD8.

Third, several groups have used antibodies to V_β to measure the influence of *MHC* haplotype on positive selection of T cells in normal, nontransgenic mice (*22, 24*). In our own studies, we have

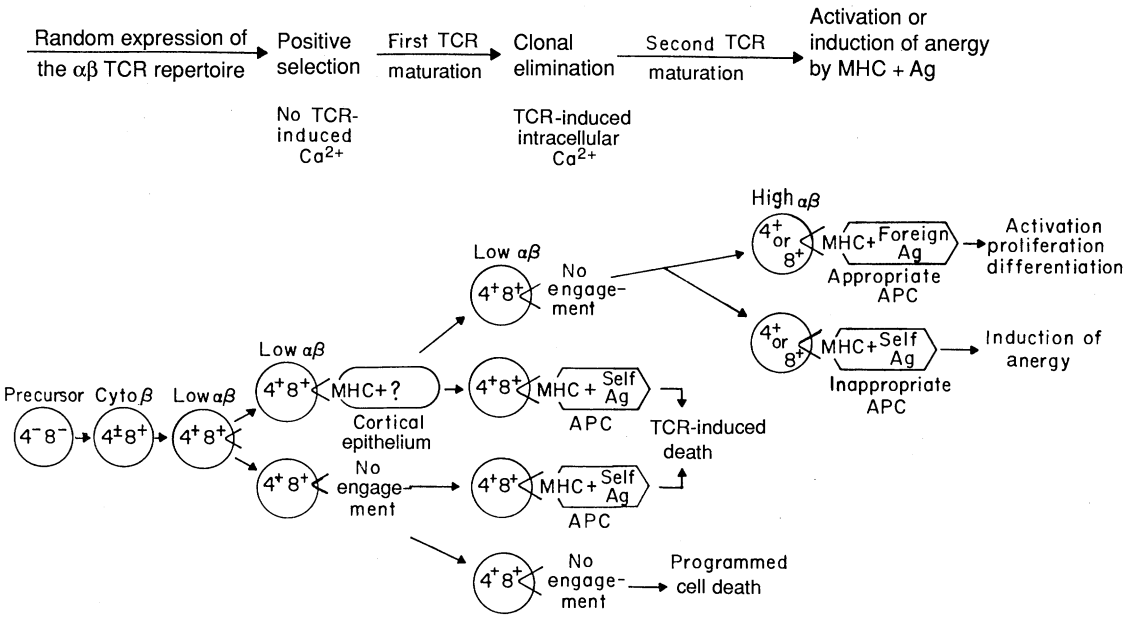

Fig. 2. The developmental pathway of mouse thymocytes. A predicted pathway for maturation of mouse thymocytes from receptor negative prothymocyte precursors to αβ TCR^+, $CD4^+$, or $CD8^+$ mature T cells is shown. The stages at which thymocytes acquire TCR and acquire or lose CD4 and CD8 are indicated. Also shown are the stages at which clonal elimination or anergy due to reaction of the thymocyte or T cell with self antigen (Ag) plus MHC occurs. The stage at which positive selection may occur is also noted. APC, antigen-presenting cell.

reproduced the classical chimera experiments, measuring positive selection of particular T cells with antibodies to V_β, rather than by peripheral reactivity (*23*). As already discussed, $V_\beta17a^+$ T cells are clonally deleted in mice that express I-E. However, in the absence of I-E expression, we found that the number of peripheral $V_\beta17a^+CD4^+$ T cells varied significantly (4 to 14%) and correlated with the *MHC* haplotype. We hypothesized that this variation in peripheral expression was a result of positive selection. We found (Fig. 3) that $V_\beta17a^+,CD4^+$ T cells are selected "better" in *MHC* haplotype *H-2*^{q} mice (Fig. 3A), that is, there are more peripheral $V_\beta17a^+$ T cells in *H-2*^{q} (14%) than in *H-2*^{b} (4%) mice. This higher expression was dominant in F_1 (b × q) mice, consistent with the idea that the level of peripheral expression was due to positive selection on *H-2*^{q}, rather than negative selection on *H-2*^{b} (Fig. 3b). Examination of expression of $V_\beta17a^+$ T cells in $F_1 \rightarrow$ parent radiation chimeras after reconstitution showed that expression levels were dictated by the *MHC* haplotype of the irradiated host rather than by that of the donor bone marrow cells, in agreement with the proposed role for radioresistant thymic epithelial cells in mediating positive selection (Fig. 3C). This was confirmed in thymic chimeras in which fetal thymus lobes from *H-2*^{b} or *H-2*^{q} mice (previously incubated in deoxyguanosine to eliminate hematopoietic elements) were grafted into thymectomized, irradiated, bone marrow–reconstituted recipients. The results (Fig. 3D) showed that the proportion of $V_\beta17a^+$ cells among the reconstituting peripheral $CD4^+$ T cells, the measure for positive selection in these experiments, was mediated by the thymic epithelium, again confirming the early results obtained in chimeric mice. In three other independent experiments examining positive selection of $V_\beta6$ or $V_\beta3$ cells by I-E, the selecting cell type was identified with I-E transgenic mice that express I-E on different cell types within the thymus. Positive selection was observed when I-E was expressed on the epithelium of the thymic cortex, but not when I-E was expressed by thymic medullary epithelial cells, again confirming the thymic cortical epithelium as the controlling cell type for positive selection (*25*).

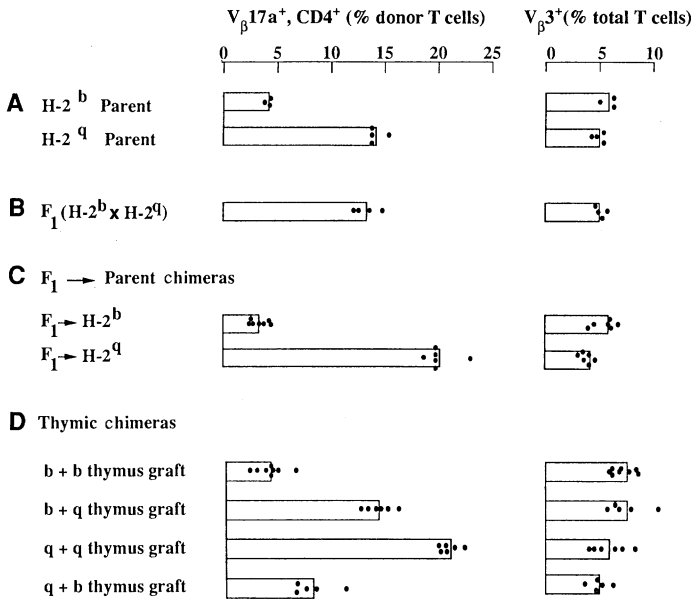

Fig. 3. Positive selection of $V_\beta17a^+,CD4^+$ T cells is controlled by MHC molecules expressed by the thymic epithelium. (**A**) The *H-2*^{b} (B10.βJ) and *H-2*^{q} (B10.QβBR) parental mice express significantly different levels of $V_\beta17a^+,CD4^+$ peripheral T cells (3.6 ± 0.3% and 14.4 ± 0.3%, respectively). (**B**) The high level of expression is dominant in F_1 (b × q) mice, which implies that the variation in levels of peripheral expression is mediated by positive selection on H-2^q, rather than negative selection on H-2^b. (**C**) Analysis of $F_1 \rightarrow$ parent chimeric mice also support a role for positive selection. The level of peripheral $V_\beta17a^+,CD4^+$ T cells in the chimeras is determined by the *MHC* haplotype of the radioresistant thymic epithelial cells of the recipient, rather than by the *MHC* of the reconstituting bone marrow–derived cells. (**D**) Analysis of levels of $V_\beta17a^+,CD4^+$ T cells in thymic chimeras confirmed the role of the thymic epithelium in positive selection. Thymectomized, irradiated, and bone marrow–reconstituted mice were grafted with fetal thymus lobes that had been treated with deoxyguanosine to remove bone marrow–derived cells. The peripheral T cells that developed in these mice expressed the level of $V_\beta17a^+,CD4^+$ T cells characteristic of the *MHC* haplotype of the thymus graft, rather than that characteristic of the *MHC* haplotype expressed by the bone marrow–reconstituting cells. These data represent a summary of experiments previously published (*17, 60*).

Although it is now clear that the MHC molecules expressed in the developing thymus exert a major influence on positive selection, there is emerging evidence for an additional influence of *non-MHC* genes (*26*). Recently, we noted that the percentages of $CD4^+$ T cells that express $V_\beta17a$ were not identical in mice that had the same or related *MHC* types, but different background genes (*27*). SWR and B10.QβBR animals, for example, are both *H-2*^{q}, and share β loci, and yet SWR animals have about 33% more $V_\beta17a^+,CD4^+$ T cells than do B10.QβBR. Our preliminary experiments strongly suggest that this is due to the effect of non-MHC, non–TCR gene products on the selection of $V_\beta17a$-bearing thymocytes (*23, 28*).

Our current understanding of positive selection is that it involves the interaction of TCR, MHC, and accessory molecules on the developing thymocyte and the thymic cortical epithelium. There also seems to be an influence of *non-MHC* background genes on positive selection, but the ligands and mechanisms involved are uncharacterized. Positive selection occurs in the thymic cortex at the immature, double-positive stage of thymocyte development, but the results of positive selection only become evident in the mature, medullary population of thymocytes. The end result is a peripheral repertoire dramatically skewed toward recognition of foreign antigen bound to self MHC.

Negative Selection

A central tenet of the immune system is self tolerance, or lack of immune responsiveness to self components. A random germline repertoire, positively selected to recognize antigen plus self MHC will certainly include autoreactive receptors. Three mechanisms have been proposed for the maintenance of self tolerance: clonal deletion, or elimination of autoreactive T cell clones; clonal anergy, or nonresponsiveness of autoreactive T cell clones; and suppression, or functional inhibition of autoreactive T cell clones. Measurement of self-reactivity in the periphery does not allow a distinction between these three mechanisms.

Clonal deletion as a major mechanism of T cell tolerance was first directly demonstrated by the absence of self-reactive clones (measured by antibodies to V_β) in mice expressing the corresponding superantigen. For example, $V_\beta17a^+$ T cells, which frequently recognize I-E, were found to be virtually absent in the periphery of I-E–expressing mouse strains (*11*). Examination of TCR expression in the thymuses of these animals showed that clonal deletion occurred during thymocyte development. Whereas there was significant expression of the autoreactive receptors in the immature thymocyte pool, expression was almost completely absent in the mature, medullary population of thymocytes. Deletion was dominant in F_1 ($V_\beta17a^+,I\text{-}E^- \times V_\beta17a^-,I\text{-}E^+$) mice.

Clonal deletion was subsequently shown in other cases of V_β-dominated recognition of superantigens. For example, T cells that express $V_\beta8.1$, $V_\beta6$, and $V_\beta9$ are eliminated in mice expressing the superantigen/MHC combination with which they can react, Mls-1^a

(the minor lymphocyte stimulating antigen–1) plus most class II alleles (*12*). $V_\beta 3$-expressing T cells are correspondingly absent from Mls-2^a– or Mls-3^a–expressing mice (*13*). Also, in addition to $V_\beta 17a$-expressing cells, $V_\beta 5$- and $V_\beta 11$-expressing cells are deleted in most I-E–expressing strains of mice (*11, 14*). In short, clonal deletion has been observed in every V_β-superantigen system described. In the case of Mls-1^a–, Mls-1^b–, and Mls-1^c–mediated deletion, the superantigen is the Mls product plus class II MHC. In the case of I-E–mediated deletion of $V_\beta 17a^+$ T cells, the superantigen includes an uncharacterized B cell product that is expressed in every mouse strain tested (*16*). In contrast, I-E–mediated deletion of $V_\beta 5$- and $V_\beta 11$-bearing T cells is dependent on strain-specific expression of a limited number of non-MHC–encoded molecules, one of which is closely linked to the endogenous provirus Mtv-9, on chromosome 12 (*29*).

Clonal deletion in the thymus has also been induced experimentally in vivo and in vitro. In vivo injection from birth of Mls-1^a (*30*) or staphylococcal enterotoxin B (*15*) or addition of toxins to fetal thymic organ cultures (*31, 32*) resulted in the efficient elimination of the mature thymocytes expressing V_βs reactive with the antigens.

Although early analyses of thymic deletion with antibodies to V_β did not demonstrate deletion at the immature thymocyte stage, accumulating evidence indicates that deletion does indeed act on these double-positive thymocytes. First, both $CD4^+$ and $CD8^+$ T cells bearing the I-E–reactive V_β, $V_\beta 17a$, are eliminated in I-E^+ mice, even though only the $CD4^+$,$V_\beta 17a^+$ population of T cells is consistently I-E–reactive in the periphery (*11, 33*). This result suggests that $CD8^+$,$V_\beta 17a^+$ T cells are eliminated at a stage when they express CD4, that is, the double-positive stage of thymocyte maturation. Second, treatment of developing thymuses with antibodies to CD4 or I-E allows the development of $CD8^+$,$V_\beta 17a$ in I-E–expressing mice (*34*). Finally, neonatal treatment of Mls-1^a–expressing mice with antibodies to CD4 resulted in the appearance of $CD8^+$,$V_\beta 6^+$ mature T cells (*35*). All these results suggest that clonal elimination can act on double-positive thymocytes.

An improved technique of measuring V_β expression on immature thymocytes as a percentage of total αβ TCR^+ thymocytes, revealed, in most cases, a reduction of approximately 50% in the immature thymocytes expressing the relevant V_β as a result of clonal deletion (*11, 31*). Thus, some, but not all, immature thymocytes are susceptible to clonal deletion. Experiments addressing the basis of this heterogeneity among immature thymocytes will be discussed below.

Clonal deletion has also been observed in the thymuses of the TCR αβ transgenic mice that express the antigen and MHC type for which the transgenic receptor is specific (*36*). The results obtained in this system at first appeared to conflict with the data obtained with normal mice, in that the transgenic mice exhibited deletion of most double-positive thymocytes, as well as the mature, single-positive thymocytes. It is likely, however, that this is the result of early expression of high-density TCR on immature thymocytes because of the preexistence of rearranged α and β genes. In TCR β-chain transgenic mice, where T cell expression on developing thymocytes follows a normal time course because surface expression must await endogenous rearrangement at the α chain locus, deletion follows the pattern seen in normal mice (*37*).

However, another possible explanation for the differences in timing of deletion in transgenic and normal mice has been proposed on the basis of studies of an αβ transgenic mouse with dual antigen specificity. The transgenic αβ TCR is specific for lymphocytic choriomeningitis virus (LCMV) plus D^b, whereas specificity for Mls-1^a is mediated by transgenic T cell receptors on the basis of their $V_\beta 8.1$-containing β chain alone (*38*). Mice that express LCMV/D^b, but not Mls-1^a, delete approximately 50% of their immature, double-positive thymocytes and all of their mature thymocytes, whereas mice that express Mls-1^a, but not LCMV, delete only their mature thymocytes. Thus, expression of different self antigens (Mls-1^a or LCMV plus D^b) causes deletion of thymocytes at different stages of development. The authors propose that the timing of deletion is determined by the affinity of the TCR for antigen. Interaction of receptors with an antigen for which they have a high affinity (LCMV + D^b) causes earlier thymocyte deletion than interaction of receptors with a low-affinity antigen (Mls-1^a). An effect of affinity is not surprising given that the number of receptors on thymocytes increases as they develop (*6*) (Fig. 2). Alternatively, however, it is possible that other factors, such as a delayed onset of Mls-1^a expression, or confinement of Mls-1^a expression to specific cells within the thymus, may account for the differential timing of deletion of Mls-1^a–reactive thymocytes.

Table 1. Interaction with bone marrow–derived cells or thymus medullary epithelium can cause clonal elimination. Donor and recipient mice were treated with rabbit antibody to mouse thymocytes to destroy recirculating T cells. Recipient animals were given 900 rads and reconstituted with 10^7 bone marrow cells from the indicated donors. Six to 8 weeks later thymocytes and T cells from these animals were examined for expression of $V_\beta 17a$, αβ, $V_\beta 2$, $V_\beta 8$, and $V_\beta 14$ as controls (not shown). Lymph node cells were also stained with anti–I-E antibodies to estimate the extent of chimerism. No detectable I-E–bearing cells were found in the lymph nodes of chimeras made by injecting 7RβL bone marrow into HTT mice and no detectable $V_\beta 8$-bearing T cells were found in the thymuses of any of these animals, indicating greater than 99% chimerism. 7RβL and HTTβL mice are $V_\beta 17a^+$, $V_\beta 8^-$. 7R and HTT mice are $V_\beta 17a^-$, $V_\beta 8^+$. Abbreviations: cort or med epi, cortical or medullary epithelial cells.

Mouse	I-E expression in cells from			Percentage of αβ thymocytes bearing $V_\beta 17a$	
	Thymus cort epi	Thymus med epi	Bone marrow cells	Immature thymocytes	Mature thymocytes
7RβL → 7R	–	–	–	10.5 ± 1.0	6.3 ± 0.8
7RβL → HTT	+	+	–	10.1 ± 0.8	1.6 ± 0.6
HTTβL → 7R	–	–	+	8.5 ± 0.2	1.0 ± 0.3
HTTβL → HTT	+	+	+	7.0 ± 0.4	1.2 ± 0.4

The identity of the cells in the thymus that are capable of inducing clonal deletion remains unresolved. It is clear that the historical distinction between the positively selecting thymic epithelium and the deleting bone marrow–derived component of the thymus is an oversimplification. Bone marrow cells appear sufficient for deletion, but are not the only cells capable of inducing deletion. This was tested in the $V_\beta 17a$/I-E system by analysis of chimeric mice constructed to express I-E on various cell types in the thymus. Mice that did or did not express I-E were irradiated and reconstituted with bone marrow cells which themselves either did or did not express I-E. Animals were thus created that expressed I-E with normal tissue distribution, or not at all, or on non–bone marrow–derived cells only, or only on bone marrow–derived cells (Table 1). Chimeras that expressed no I-E at all contained immature thymocytes of which about 10% bore $V_\beta 17a$. Fewer mature thymocytes (6%) in these animals bore $V_\beta 17a$, presumably because $V_\beta 17a$-containing receptors are relatively poorly positively selected in *H-2*^{s} mice, the *H-2* haplotype used in this experiment. As predicted by classical experiments, expression of I-E by bone marrow–derived cells led to good tolerance to I-E, $V_\beta 17a$ cells were almost eliminated from the mature thymocytes of mouse strain HTTβL → 7R animals. Somewhat surprisingly, such animals also had reduced numbers of immature, cortical thymocytes bearing $V_\beta 17a$ by comparison with I-E–negative controls, demonstrating that bone marrow–derived cells, macrophages, B cells, or dendritic cells in the thymus cortex could cause clonal elimination of some immature thymocytes. Al-

so, the interaction of receptors on thymocytes with antigen-MHC on thymus medullary epithelial cells also led to elimination. 7RβL → HTT animals, for example, that expressed I-E on thymus epithelial cells only, contained almost the same frequency of immature $V_\beta 17a^+$ thymocytes as controls although the frequency of mature, medullary thymocytes bearing $V_\beta 17a$ was drastically reduced. Data from I-E transgenic animals supported these conclusions (*39*).

The matter of whether interaction of thymocyte receptors with antigen-MHC on thymus cortical epithelial cells can result in clonal elimination is more difficult to resolve. 7RβL → HTT chimeras do not have significantly lower percentages of immature, cortical $V_\beta 17a$-bearing thymocytes than controls. This could be because interaction of thymocyte receptors with ligands on thymus cortical epithelial cells is intrinsically unable to lead to thymocyte death, or it may be due to poor expression of the $V_\beta 17a$-engaging ligand on thymus cortical epithelial cells, a distinct possibility since $V_\beta 17a$-bearing T cells are specific for I-E plus an unknown B cell product (*16*), a combination known to be not well expressed on thymus cortical epithelial cells (*40*).

In summary, these data show that tolerance can be induced in thymocytes by engagement of their receptors with ligands expressed on bone marrow–derived cells in either the thymus cortex or medulla. Clonal elimination can also occur as a result of thymocyte receptor engagement by ligands on thymus medullary epithelium. The ability of ligands on thymus cortical epithelial cells to induce thymocyte elimination was not completely tested in these experiments, although it remains an important matter, as discussed below.

The Paradox—What, How, Where, or When?

The role of the αβ TCR in both positive and negative selection of thymocytes, as well as in T cell activation in the periphery, has been clearly established. However, we do not understand how ligation of the same molecule (TCR) can result in such dramatically different responses, such as maturation (positive thymic selection), death (negative thymic selection), or activation (peripheral effector function). Why is the repertoire of receptors selected on self MHC plus self peptide (*40a*) and subsequently tolerized on self MHC plus self peptide not completely overlapping, resulting in no surviving cells to constitute the peripheral repertoire? Two hypotheses have been proposed, one based on affinity arguments, and a second based on the idea that MHC molecules on thymus cortical epithelial cells may not be identical to those found elsewhere.

The affinity hypothesis proposes that whereas thymocytes with both high- and low-affinity receptors are positively selected on self MHC, only the high-affinity clones are subsequently deleted (*10*). Thus, the clones that have low affinity for self survive deletion and in the periphery constitute the repertoire for foreign peptides bound to self MHC. This hypothesis has the advantage that it does not invoke any novel properties for the selecting cortical epithelial cell, other than an ability to engage thymocytes expressing receptors with low affinities for self MHC. This, of course is also a disadvantage since we have no idea what the structural basis for such an ability might be. Perhaps thymus cortical epithelial cells bear high densities of "accessory ligands," proteins other than the receptor and MHC that serve to promote the interaction of thymocytes with these cells.

The "altered ligand" hypothesis was prompted by the solution of the structure of a class I MHC molecule, and the finding that this molecule contains a peptide binding groove, which will probably always be occupied, if not by foreign peptides, then by peptides derived from self (*1*). Thus, it is possible that many variations of self MHC can be created, by binding of many different self peptides. Various forms of the altered ligand hypothesis have been proposed (*41*), but all rest on the idea that thymus cortical epithelial cells may express MHC molecules bound to a collection of peptides that are not found elsewhere in the animal. If such cells cannot induce clonal elimination (the available experimental data are equivocal on this point, discussed above) then this idea explains how thymocyte positive and negative selection can both occur, and lead to the production of mature T cells able to recognize antigen/self MHC.

A few experiments support the idea that MHC on thymus cortical epithelial cells is not exactly like that expressed elsewhere. First, an antibody has been produced against a unique I-A determinant, which depends on a molecular contribution from I-E. This antibody binds to about 15% of the I-A molecules on peripheral tissues, but does not engage I-A on thymus cortical epithelial cells (*42*). Second, we have shown that some T cell hybridomas, specific for antigen plus self MHC, can react, weakly, with self MHC expressed by thymus cortical epithelium, but not by other cells (*40*).

Both of these hypotheses require that the developing thymocyte be able to distinguish between receptor interactions inducing positive selection, provided by thymus cortical epithelial cells, and those inducing clonal elimination. The first, affinity, theory deals with this problem by suggesting that very weak engagement is positively selective, and stronger engagement is lethal. The second theory requires that interaction of thymocyte receptors and thymus cortical epithelial cells in some way delivers a different signal to the thymocyte than does interraction of its receptors with ligands on any other cell type. This could be because thymus cortical epithelial cells make fewer, or a different set of accessory signals, for example, lymphokines, than other cells, an idea for which there is some evidence (*43*). Alternatively, this could be due to the differentiative state of the thymocyte when it is most exposed to thymus cortical epithelial cells. Again, this is an idea for which there is some evidence since we have shown that receptor-bearing immature thymocytes exist in two stages, based on their sensitivity to clonal elimination. It is thought that clonal elimination in thymocytes is caused by receptor engagement, which leads to increased levels of free intracellular Ca^{2+}, a signal thought to be suicidal for immature thymocytes (*44*). We have shown that immature thymocytes exist in two stages. At one of these, receptor engagement does not cause increases in intracellular Ca^{2+}, and such cells do not die when they bind antigen. At another, probably later, stage, receptor engagement does lead to higher intracellular Ca^{2+} and death (*31*). The feature that distinguishes these two stages seems to be the coupling of the αβ receptor to CD3, as anti-CD3 antibodies caused increased intracellular Ca^{2+} and death in both populations of immature thymocytes (*31, 44*). Given that these two populations of immature thymocytes exist, it might be that the first population, which cannot be killed by receptor engagement, is the population on which the forces of positive selection act.

In summary, the paradox of positive selection and clonal elimination of thymocytes by what appears to be the same interaction is not resolved. Factors such as what form of antigen is recognized (altered ligand?), how the developing thymocytes bind antigen (affinity differences?), where in the thymus antigen is encountered and in association with what antigen-presenting cells (cortex or medulla? thymic epithelial cells or bone marrow–derived cells?), and when the developing thymocyte encounters antigen (state of thymocyte maturation?) may all play a role.

Is Clonal Deletion All There Is?

Although it is now well established that clonal deletion is a major mechanism of tolerance for self antigens that are expressed in the

thymus, the question arises as to how tolerance to self antigens that are not constituitively expressed in the thymus is accomplished. Several possibilities can be suggested. First, perhaps clonal deletion can also act in the periphery on mature T cells. However, several lines of evidence argue against this possibility. Intravenous injection of Mls-1^a–bearing splenocytes into adult mice does not cause elimination of Mls-1^a–reactive $V_\beta 6$- and $V_\beta 8.1$-bearing T cells (*44, 45*). Also, neonatal thymectomy prevents clonal deletion of self-reactive $V_\beta 11^+$ T cells from I-E–expressing mice (*47*). In addition, there is no evidence for clonal deletion of $V_\beta 3$ and $V_\beta 11$ cells in nude mice expressing the corresponding self antigens [Mls-2^a or 3^a and I-E, respectively (*48*)]. Finally, binding of TCR with MAb or exposure of cells to Ca^{2+} ionophores kills certain populations of thymocytes but rarely kills peripheral T cells, suggesting that susceptibility to clonal elimination is restricted to a stage in T cells' lives that occurs in the thymus.

Second, it has been suggested that the restricted cell-type expression of class II MHC molecules, coupled with the central role of $CD4^+$ class II–restricted T cells in the induction of peripheral B and T cell responses, renders peripheral mechanisms of tolerance unnecessary because peripheral presentation of self molecules by cells lacking class II expression, or by class II expression in the absence of costimulatory signals provided by "proper" antigen-presenting cells, would not activate self-reactive $CD4^+$ T cells (*49*). This predicts that induction of class II expression in these peripheral cells, for example, as a result of γ-interferon production during infection may lead to antigen presentation and bystander lymphokine production resulting in autoimmunity, a phenomenon that is sometimes observed, at least transiently.

A third possibility is that self antigens that are not anatomically expressed in the thymus enter the circulation and are "captured" and presented by resident thymic or circulating antigen-presenting cells, resulting in a normal scenario for thymic clonal deletion. In one study, intravenously injected myoglobin was presented by class II–expressing dendritic cells in the thymus as soon as 15 min after injection (*50*). If all peripheral antigens could be efficiently expressed in the thymus by this mechanism, thymic clonal deletion would be a sufficient mechanism for tolerance. Although this might be an adequate explanation for class II presentation of exogenously acquired antigens, it has been shown that class I antigen presentation is restricted to endogenously synthesized molecules (*51*). Therefore peptide fragments of proteins made by nonrecirculating class I^+ cells cannot be picked up by class I molecules on circulating macrophages and dendritic cells and carried to the thymus to induce clonal elimination. However, if tolerance need only be established to class II–reactive T cells, as discussed above, presentation of class II–restricted antigens would be sufficient.

Fourth, nondeletional mechanisms of peripheral tolerance, such as clonal inactivation (anergy) or suppression may operate. A discussion of the role of suppression in T cell tolerance is beyond the scope of this review, and the role of clonal anergy is presented in detail (Ramsdell and Fowlkes) in this issue (*52*). However, several recent reports have described anergy as a mechanism of tolerance in situations where the anatomical expression of self antigens has been experimentally manipulated. For example, intravenous injection of Mls-1^a–bearing spleen cells into adult *Mls-1^b* mice does not cause $V_\beta 6$-bearing (Mls-1^a–reactive) T cells to disappear, but it does render them nonresponsive (anergic) to challenge (*46*). Abnormal expression of I-E on peripheral cells (acinar cells of the pancreas) in transgenic mice has the same effect on I-E–reactive $V_\beta 11$- and $V_\beta 17a$-bearing T cells (*53*). Also, manipulation of thymic expression of Mls-1^a and I-E in chimeric mice results in anergy of $V_\beta 6$- and $V_\beta 17a$-bearing T cells, respectively (*54*). These studies and another well-characterized in vitro system of anergy [(*55*), discussed in detail

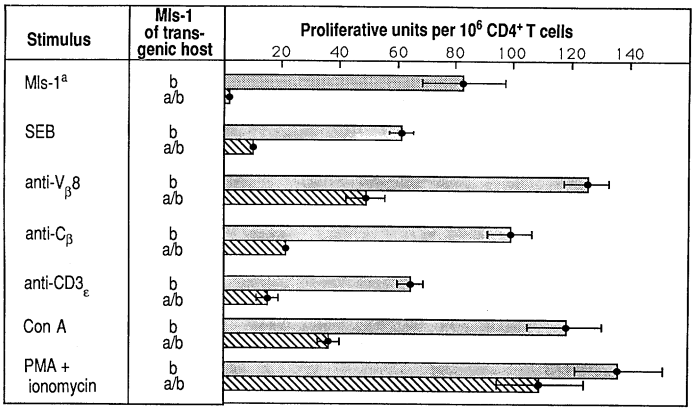

Fig. 4. Mls-1^a induces anergy in some peripheral T cells in mice expressing a $V_\beta 8.1^+$ transgenic TCR β chain gene. The $V_\beta 8.1^+$ TCR β chain transgenic mouse was bred with (nontransgenic) mice expressing Mls-1^a or Mls-1^b. Peripheral T cells from the two lines of mice were assessed for their in vitro proliferation to a variety of stimuli. The results are presented as arbitrary proliferative units calculated from the slopes of the dose-response curves. As shown, the proliferation induced by stimulation via the TCR or CD3 was reduced >50% in the Mls-1^a–expressing TCR β chain transgenic mice. However, stimulation with PMA and ionomycin, stimuli that bypass the TCR and CD3, resulted in comparable levels of T cell proliferation, indicating that approximately 50% of the peripheral T cells in the Mls-1^a–expressing transgenic mice were anergic (*58*).

elsewhere in this issue (*56*)], suggest that binding of MAb to TCR in the absence of as yet undefined costimulatory signals contributed by the antigen-presenting cell may result in clonal inactivation rather than responsiveness. This theory has been tested in vitro for the anergy induced by I-E expression on pancreatic cells in transgenic mice, mentioned above. These cells are unable to stimulate T lymphocytes reactive to I-E plus peptide and, in fact, render the T cells anergic to subsequent stimulation by proper antigen-presenting cells (*57*).

We also have studied the role of clonal anergy in T cell tolerance (*58*). We generated a mouse transgenic for a TCR $V_\beta 8.1$ β chain cloned from a strongly Mls-1^a–reactive T cell hybridoma. The mouse was then bred with Mls-1^a– and Mls-1^b–expressing strains of mice to examine the mechanism of tolerance. Surprisingly, despite the fact that clonal deletion has been shown to be a major mechanism of tolerance of $V_\beta 8.1$-bearing T cells to Mls-1^a in normal mice (*12*) as well as another $V_\beta 8.1$ transgenic mouse (*39*), we failed to see dramatic clonal deletion in our Mls-1^a–expressing transgenic animals. However, the mouse was tolerant to Mls-1^a. Furthermore, examination of peripheral T cell reactivity showed significantly reduced proliferation (compared with the corresponding Mls-1^b transgenic mice) in response to antibodies to TCR and CD3, as well as to concanavalin A and the foreign superantigen, staphylococcal enterotoxin B. However, the cells were capable of responding to stimulation with phorbol ester and ionomycin (Fig. 4). Examination of the calcium response of these cells after binding of MAb to the TCR revealed that they failed to increase their level of intracellular calcium (*59*).

Our experiments therefore show that whereas tolerance to Mls-1^a among T cells bearing $V_\beta 8.1$ and a variety of J_βs, V_αs, and so on, in normal mice is mediated predominately by clonal deletion, tolerance in a population of T cells bearing a single $V_\beta 8.1$-including β chain in these particular transgenic mice is mediated predominantly by clonal anergy. T cell development and TCR and accessory molecule density are normal during early ontogeny in both the Mls-1^a– and Mls-1^b–expressing transgenic mice. In addition, Mls-1^a expression in the thymus appears to be normal because deletion of $V_\beta 8.1$- and $V_\beta 6$-expressing T cells occurs normally in the Mls-1^a nontransgenic

littermates. Thus, it is likely that the difference between normal mice and our transgenic mice is controlled by receptor affinity for Mls-1^a and that the population of TCRs generated from the transgenic β chain has on the average a low affinity for Mls-1^a. This is supported by the finding that the Mls-1^a reactivity of T cells bearing this particular β chain, in contrast to most $V_β$8.1-bearing T cells, appears to be very much dependent on contributions from the α chain, since approximately half of the β-transgene–expressing T cells in these mice cannot recognize Mls-1^a (*59*).

Summary

Although immature thymocytes bearing many combinations of TCR α and β variable components are produced, by no means all of these combinations are expressed on the mature T cells of any given individual. The receptor repertoire of mature T cells is limited by two apparently contradictory processes that occur in the thymus, positive selection and clonal elimination. The paradoxical existence of these two processes has yet to be explained experimentally, although it can be accommodated by several hypotheses.

Self tolerance is crucial to survival. Therefore, the immune system has evolved at least two methods of ensuring that αβ TCR-bearing T cells are not reactive to self, clonal elimination in the thymus and anergy induced in the periphery. We do not now understand completely why some self antigens induce tolerance by one mechanism or the other. We also do not know why some potentially autoimmune T cells slip through these two traps to escape and give rise, at a later date, to autoimmune disease.

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60. The *H-2^b* and *H-2^q* parental mice were B10.βJ and B10.QβBR mice, respectively. Donors and recipients for F1 → parent chimeras were (B10.βJ × B10.QβBR)F_1 and B10 or B10.Q animals. Thymus chimeras were created using thymuses from C57BL/6 or B10.Q donors and bone marrow from C57L or SWR; recipients were thymectomized B6.Lyt1.1 or B10.Q mice. In all cases donor and recipient mice were pretreated with rabbit antiserum to mouse thymocyte, and recipients were irradiated with 750 R before transfer of about 10^7 bone marrow cells of the indicated type. Where indicated, 1 week after irradiation, thymectomized mice were grafted with fetal thymus lobes that had been incubated with deoxyguanosine to remove bone marrow–derived cells. Chimeras were analyzed for percent T cell expression of $V_β$'s by standard techniques (*11*). Extent of chimerism was measured with antibodies to MHC, $V_β$, Lgp100, and Ly1 as described (*17*).
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