(*29*), and 20 μl was subjected to electrophoresis in 10% SDS-polyacrylamide gels, electroblotted, and probed with crude antiserum to ABF1 (1:500 dilution). To assay for ABF1-specific DNA binding activity in *E. coli* extracts, we plated 5×10^4 λABF1 on small petri dishes. After plaques first appeared (3 to 4 hours), 3 ml of TN was placed on the dishes and they were incubated for 4 hours at 37°C. Buffer was harvested, centrifuged, and assayed for DNA binding in a gel retention assay with either the 275-bp Bam HI–Sal I fragment from pBR322 (lanes 1 to 4) or the 325-bp Eco RI–Hind III fragment from pARS1.2 (lanes 5 to 8) as described (*3*). All yeast techniques were from *Methods in Yeast Genetics* [F. Sherman, G. R. Fink, J. B. Hicks (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986)].

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A Nondeletional Mechanism of Thymic Self Tolerance

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T cells become tolerant of self antigens during their development in the thymus. Clonal deletion of thymocytes bearing self-reactive T cell receptors is a major mechanism for generating tolerance and occurs readily for antigens expressed by bone marrow–derived cells. Tolerance to antigens expressed on the radioresistant thymic stromal elements is demonstrated here to occur via a nondeletional mechanism. For minor lymphocyte stimulatory (Mls-1^a) and major histocompatibility complex (MHC) antigens, this alternate form of tolerance induction results in clonal anergy.

IT IS OUR CURRENT VIEW THAT A PRECURSOR T cell entering the thymus will ultimately undergo one of three developmental fates. An immature T cell that does not recognize any component of the thymic stroma will die, presumably via a type of programmed cell death (*1*). An interaction between the thymocyte T cell receptor (TCR) and MHC molecules (or MHC plus peptide) on a radioresistant epithelial element rescues the cell from death and allows differentiation to a functionally mature T cell (positive selection) (*2–4*). In contrast, interactions with bone marrow–derived elements such as macrophages or dendritic cells result in clonal deletion of the self-reactive thymocyte (*5–7*).

Our understanding of thymic tolerance was enhanced by the development of monoclonal antibodies (MAbs) to the particular TCR V_β regions that confer specificity for defined antigens (*8–10*). These MAbs were used to show that clonal deletion of self-reactive cells is a major mechanism for maintaining tolerance to self antigens. This process also occurs in transgenic mice in which the majority of T cells bear a single receptor with autospecificity (*11*).

Deletion occurs at a $CD4^+8^+$ precursor stage of development and involves the CD4 and CD8 accessory molecules (*12*). Although there is evidence that clonal deletion occurs via an interaction with bone marrow–derived cells, it remains unclear whether epithelial elements within the thymus can also promote this event. In radiation bone marrow chimeras, donor T cells may develop functional tolerance to radioresistant thymic epithelial antigens (*3, 13*). We now investigate the mechanism by which the radioresistant elements within the thymus can induce self tolerance.

To manipulate the site of antigen expression, a series of radiation bone marrow chimeras were constructed such that the antigens recognized by specific V_β's were expressed only by the radioresistant host cells and not by bone marrow–derived elements. All of the chimeras were of the parent bone marrow into F_1-irradiated host type ($P \rightarrow F_1$; H-2^s or H-$2^k \rightarrow$ H-$2^{s\times k}$) in which the host (B10.S × AKR)F_1 was always held constant. This host expresses the MHC haplotype H-$2^{s\times k}$, and the minor lymphocyte stimulatory (Mls) antigens, Mls-$1^{a\times b}$. The donor was derived from either H-2^s or H-2^k strains that were Mls-1^b. In all cases the donor and host could be distinguished by their expression of either CD5 or CD45 alleles.

Initially we analyzed the chimeras for clonal deletion of $V_\beta 6$-bearing T cells. T cells bearing $V_\beta 6$ are deleted in Mls-1^a mice if these mice express certain MHC haplotypes such as H-2^k and H-2^d (such haplotypes are referred to as "permissive" for Mls presentation) (*10*). To increase the reliability of the analysis, we enriched thymocytes for mature cells by J11d MAb (*14*) plus complement lysis. This treatment enriches for $CD3^{Hi}$ mature cells (>90%). Two-color flow cytometric (FC) analyses of $V_\beta 6$ versus CD4 or CD8 were performed, and the resulting data were normalized for the number of $CD3^{Hi}$, $CD4^+8^-$, or $CD4^-8^+$ cells (an example of which is in Fig. 1). $V_\beta 6$ cells were present in SJL (H-2^s) mice (Fig. 1A), but deleted in both the $CD4^+8^-$ and

Table 1. Analysis for clonal deletion of $V_\beta 6$ thymocytes in chimeras made with an Mls-1^a–bearing host. Bone marrow chimeras were constructed as in Fig. 1. The (B10.S × AKR)F_1 host is Mls-$1^{a\times b}$ and all donors are Mls-1^b except CBA/J, which is Mls-1^a. This latter chimera serves as a control to indicate the $V_\beta 6$ frequency when the antigen relevant for deletion is on both the host and donor. The FC analysis was performed 35 to 80 days after reconstitution when chimeras were >97% donor type. For FC analysis, thymocytes from individual mice were treated with J11d.2 plus complement to enrich for mature cells. Remaining cells were stained as in Fig. 1. Percentages represent the arithmetic mean and SEM for three to eight individual animals. All values have been normalized to include only $CD3^{Hi}$ cells.

Strains	$V_\beta 6$ thymocytes (%)		
	Total*	$CD4^+8^-$†	$CD4^-8^+$‡
	H-2^s donors		
SJL → (B10.S × AKR)F_1	9.7 (±1.9)	10.1 (±2.0)	8.2 (±1.2)
A.SW → (B10.S × AKR)F_1	5.9 (±0.5)	5.7 (±0.6)	7.2 (±0.8)
SJL	10.6 (±0.6)	8.8 (±0.3)	14.2 (±0.6)
A.SW	7.2 (±0.4)	6.1 (±0.5)	11.2 (±1.5)
(B10.S × AKR)F_1	0.2 (±0.1)	0.1 (±0.1)	0.3 (±0.3)
(SJL × AKR)F_1	0.9 (±0.3)	0.9 (±0.3)	0.6 (±0.3)
	H-2^k donors		
CBA/J → (B10.S × AKR)F_1	0.7 (±0.3)	0.7 (±0.1)	1.9 (±1.5)
CBA/Ca → (B10.S × AKR)F_1	0.5 (±0.3)	0.3 (±0.4)	0.7 (±0.7)
C3H → (B10.S × AKR)F_1	2.2 (±1.0)	2.3 (±1.1)	2.0 (±1.2)
CBA/J	0.6 (±0.3)	0.5 (±0.1)	0.8 (±0.2)
CBA/Ca	10.2 (±1.2)	8.8 (±1.3)	14.5 (±0.6)
C3H	12.2 (±0.6)	9.8 (±1.7)	16.4 (±4.1)

*Values represent total $V_\beta 6^+$ cells divided by total $CD4^-8^+$ (×100). †Values represent $V_\beta 6^+$, $CD4^+8^-$ cells divided by total $CD4^+8^-$ (×100). ‡Values represent $V_\beta 6^+$, $CD4^-8^+$ cells divided by total $CD4^-8^+$ (×100).

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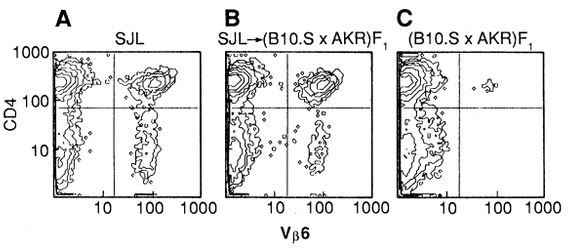

Fig. 1. Analysis of $V_\beta6$ expression by $CD4^+8^-$ and $CD4^-8^+$ thymocyte subsets in normal donor (**A**), $P \rightarrow F_1$ chimera (**B**), or normal host (**C**). SJL mice were obtained from the National Cancer Institute, National Institues of Health. $(B10.S \times AKR)F_1$ mice were bred in our facility. Bone marrow chimeras were constructed by 1000-R gamma irradiation (Cs source) of $(B10.S \times AKR)F_1$ mice followed by intravenous injection of 1×10^7 to 2×10^7 T-depleted bone marrow cells 12 to 24 hours later. $(B10.S \times AKR)F_1$ mice were maintained in sterile cages and on antibiotic water (Biosol, Upjohn) throughout the study. T cell depletions were done with a mixture of MAbs to Thy-1 [J1J (*28*) or T11 D7e (Bioproducts for Science)] and CD5 (C3P0) (*14*) and complement (Low Tox-M, Cederlane). FC analysis was performed 35 to 80 days after reconstitution, when chimeras were >97% donor type. For FC analysis, thymocytes were treated with J11d.2 (*14*) plus complement to enrich for mature T cells (>90% $CD3^{Hi}$). The remaining cells were stained with a MAb to $V_\beta6$ (RR4-7) (*29*) and fluorescein isothiocyanate (FITC)–labeled goat antibodies to rat immunoglobulin G (IgG) (Kirkegard and Perry), then a biotinylated MAb to CD4 (H129.19) (*30*) or CD8 (Becton Dickinson; not shown in figure) and allophycocyanin-avidin (Caltag). Cells were analyzed on a FACS 440.

Table 2. $V_\beta17a^+$ thymocytes are not deleted in SJL → $(B10.S \times AKR)F_1$ chimeras. $J11d^-$ thymocytes were prepared as in the legend to Fig. 1 and analyzed by two-color FC for $V_\beta17a$ versus CD4 or CD8. Anti-$V_\beta17a$ (KJ23) binding was detected with FITC-labeled goat antibody to mouse IgG2a (Fisher) and counterstained for CD4 and CD8 as in Fig. 1. Chimeras were >95% donor type. Percentages represent arithmetic mean and SEM for three to eight individual animals, and values are normalized to $CD3^{Hi}$ cells.

Strains	$V_\beta17a$ Thymocytes (%)		
	Total*	$CD4^+8^-$†	$CD4^-8^+$‡
SJL → $(B10.S \times AKR)F_1$	7.4 (±0.7)	8.0 (±0.8)	5.0 (±1.0)
SJL	10.4 (±0.4)	12.3 (±1.5)	7.5 (±0.9)
$(SJL \times AKR)F_1$	2.1 (±0.9)	2.3 (±1.1)	1.6 (±0.6)

*Values represent total $V_\beta17^+$ cells divided by total $CD4^+8^-$ + $CD4^-8^+$ (×100). †Values represent total $V_\beta17^+$, $CD4^+8^-$ cells divided by total $CD4^+8^-$ (×100). ‡Values represent total $V_\beta17^+$, $CD4^-8^+$ cells divided by total $CD4^-8^+$ (×100).

$CD4^-8^+$ T cell subpopulations in normal $(B10.S \times AKR)F_1$ mice (Fig. 1C). However, $V_\beta6$ was not deleted in bone marrow chimeras with this F_1 as the recipient when the donor was SJL (Fig. 1B).

In a series of similar chimeras, thymocytes and lymph node cells were analyzed for $V_\beta6$ (Table 1); however, since the results were comparable, only the thymus data are shown (*15*). Whenever the donor was of the $H\text{-}2^s$ haplotype, little or no deletion of $V_\beta6$ thymocytes occurred in the $CD4^+8^-$ subset. In contrast, when the donor was $H\text{-}2^k$, deletion of $V_\beta6$ cells was extensive in both the CD4 and CD8 subsets. The host was the same in all of these chimeras; therefore, the ability to cause deletion was a function of the bone marrow donor rather than the host. Because the relevant Mls antigen was contributed only by the host, these observations are consistent with previous findings that the $Mls\text{-}1^a$ antigen can be expressed by radioresistant cells in the host and transferred to donor-derived cells that mediate deletion, whenever the donor expresses an appropriate MHC haplotype (*16*). As deletion was good with $H\text{-}2^k$ donors, yet poor with $H\text{-}2^s$ donors (using the same host), the radioresistant thymus that expressed $H\text{-}2^{s\times k}$ and had the relevant self antigen was, therefore, poor at inducing clonal deletion (*17, 18*). It is unknown at the present time whether the Mls antigen was from the thymic epithelium or from some other source within the irradiated host. In this regard, no B cells of host type were detected in the chimeras. This issue of whether the thymus provides the Mls, however, can be addressed by thymus-grafting experiments.

T cells bearing $V_\beta17$ were also analyzed in the SJL → $(B10.S \times AKR)F_1$ chimera (Table 2). This TCR V_β is specific for MHC class II, I-E molecules (*8, 19*), which in the chimera are expressed only by the $(B10.S \times AKR)F_1$ host. Most $V_\beta17$-bearing cells (±70%) were not deleted in the chimera, whereas >80% of $V_\beta17^+$ cells are deleted in the $(SJL \times AKR)F_1$. Similar results were obtained by thymus grafting experiments (*7*). There are several possible reasons that the frequency of $V_\beta17^+$ cells is lower in the chimera compared to a normal SJL. There are differences in the positive selecting elements, which are associated with the thymic epithelium, and could therefore affect the $V_\beta17$ frequency. Not all $V_\beta17^+$ cells are specific for I-E (*20*), and therefore deletion may be mediated by other host-derived antigens presented in the context of donor MHC. Finally, it is possible that the thymic epithelium was causing a partial deletion of $V_\beta17^+$ cells (perhaps only of high-affinity clones). If this decrease does reflect deletion mediated by the epithelium, it is an inefficient process relative to that mediated by bone marrow–derived elements. This was confirmed in the mixed bone marrow chimera SJL + $(B10.S \times AKR)F_1 \rightarrow (B10.S \times AKR)F_1$. In chimeras of this type, in which the F_1 donor–derived cells make up only 20% of total donor cells, the frequency of $V_\beta17^+$ cells was similar to the $(SJL \times AKR)F_1$ controls (*21*). As was the case for $V_\beta6$, the data for $V_\beta17$ suggests that the thymic epithelium is poor at inducing clonal deletion of self-reactive thymocytes.

The SJL → F_1 chimeras appeared healthy and survived with no spontaneous deaths or evidence of disease. However, since substantial numbers of $V_\beta6$- and $V_\beta17$-bearing T cells were not deleted in SJL → $(B10.S \times AKR)F_1$ chimeras, we determined whether these mice were functionally tolerant of the relevant host antigens, $Mls\text{-}1^a$ and I-E. The capacity of T cells from these chimeras to respond to F_1 host cells was tested in a mixed lymphocyte response (MLR) assay (Fig. 2). Whereas cells from the chimera responded vigorously to a third party control, they failed to respond to the $(B10.S \times AKR)F_1$ stimulators (as well as to normal SJL). Both lymph node cells (Fig. 2B) and mature thymocytes (Fig. 2D) were nonresponsive to the host-type stimulators, whereas the corresponding populations of T cells from a normal SJL responded well (Fig. 2, A and C). These results suggest the donor-derived T cells were tolerant of host antigens.

These data are in contrast with previous studies involving thymus grafts into nude mice in which only incomplete tolerance was observed (*5, 6*). In these earlier experiments, the thymus grafts were depleted of host bone marrow–derived cells with deoxyguanosine before transplantation. Cells from the grafted mice showed little or no tolerance of the graft MHC type in an MLR, despite maintaining the thymus in vivo. In certain other $P \rightarrow F_1$ chimeras involving high irradiation of the host, tolerance was observed for $CD4^-8^+$ but not $CD4^+8^-$ cells (*22*). Whether these qualitative differences in the extent of tolerance reflect differences in the methods used to generate the chimeric situation or some other aspect of the experimental procedure is unclear at present.

Therefore, to test the tolerant status of the chimeric cells in vivo, in an environment that might prime any cells with host reactivity, normal $(B10.S \times AKR)F_1$ mice were

irradiated and injected with lymph node cells from an SJL → (B10.S ×AKR)F_1 chimera (Fig. 3). Transfer of normal SJL, but not chimeric, lymph node cells resulted in the death of the recipient, most likely from graft versus host disease (GVHD). The chimeric cells were functional in this assay, as they could kill a third party (DBA/2) recipient. The failure to elicit a lethal GVHD by the chimeric cells shows that they were specifically tolerized to all host antigens in the chimera and are resistant to priming.

Since T cells from the chimera were tolerant of self antigens of host origin, but little clonal deletion had occurred, we determined whether cells bearing the relevant specific V_β's were able to undergo TCR-mediated activation. In other studies, purified $CD4^+8^-$ or $CD4^-8^+$ cells proliferate in response to immobilized MAbs to TCR (*21*). Thus, lymph node cells or $J11d^-$ thymocytes were stimulated with MAbs to $V_\beta6$ or $V_\beta17$ on microtiter plates (Fig. 4). Both the SJL and the SJL → (B10.S × AKR)F_1 chimeric thymocytes responded vigorously to the H57-597 MAb (*23*) that recognizes all αβ TCR cells (Fig. 4A). In individual

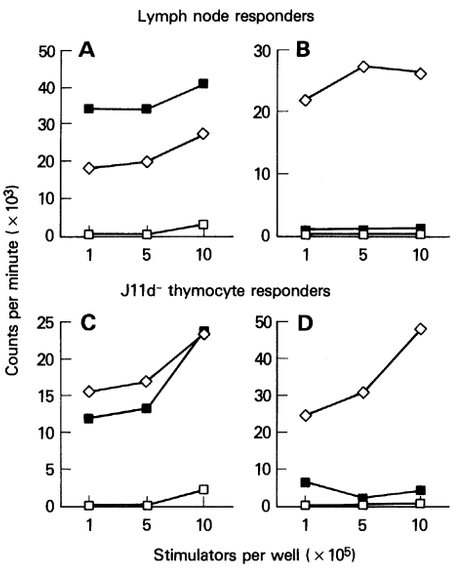

Fig. 2. SJL → (B10.S × AKR)F_1 chimeras are tolerant of F_1 hosts in an MLR. Cells from SJL (**A** and **C**) or SJL → F_1 chimeras (**B** and **D**) were plated (2×10^5 per well) in round-bottomed 96-well plates. Responders (>95% donor type) were either whole lymph node cells (A and B) or $J11d^-$ thymocytes (C and D). Stimulators were 3000-R, T-depleted spleen cells derived from B6D2 (◇), (B10.S × AKR)F_1 (■), or SJL (□). Stimulators were added, the plates were incubated at 37°C for 4 days in complete medium [RPMI 1640 plus fetal calf serum (10%)], and then the cells were pulsed with 1 μCi of [^{3}H]thymidine for an additional 12 to 18 hours. Samples were processed for standard scintillation counting. Data represent one of three similar experiments. In additional experiments with other third party controls (DBA/2, BALB/c, and C57Bl/6), comparable results were obtained.

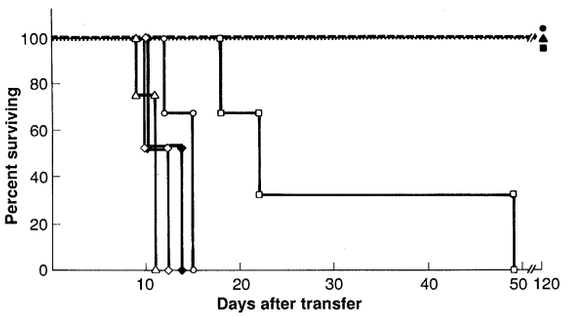

Fig. 3. P → F_1 chimeric lymph node cells do not elicit a graft-versus-host response in secondary F_1 hosts. (B10.S × AKR)F_1 (triangles, circles, squares) or DBA/2 (diamonds) mice were irradiated 600 R (squares) or 750 R (triangles, diamonds, circles) and injected with lymph node cells (0.6×10^7 to 1.5×10^7) from SJL (open symbols) or SJL → F_1 (closed symbols) donors. All mice were maintained in sterile cages and on antibiotic water throughout the study. Analysis of samples from (B10.S × AKR)F_1 recipients that had received chimeric lymph node cells demonstrated the presence of donor-type cells >100 days after transfer.

experiments, the response to this MAb varied slightly in magnitude between SJL and chimeric mice; nevertheless, the concentration dependence was qualitatively similar. In contrast, the chimeras (either lymph node or $J11d^-$ thymocytes) always responded poorly to antibody to $V_\beta17$ (anti-$V_\beta17$) or antibody to $V_\beta6$ (anti-$V_\beta6$) (Fig. 4, B and C) (*21*). At the highest stimulating doses of anti-$V_\beta17$, the chimeras did proliferate, perhaps because not all $V_\beta17^+$ cells are specific for I-E (*20*). This explanation is supported by the incomplete deletion of $V_\beta17$ in the (SJL × AKR)F_1 (Table 2). Nonetheless, the response to $V_\beta17$ in the chimeric mice demonstrates a significant shift (100 times) in the concentration dependence.

We next explored the mechanism of the T cell anergy induced by thymic stromal elements. Under certain circumstances helper T cell clones can be rendered unresponsive because of an inability to produce interleukin-2 (IL-2), despite the expression of functional IL-2 receptors in response to TCR occupancy (*24*). Tolerized peripheral T cells also express IL-2 receptors in response to antigen (*25*). When we stimulated $V_\beta17^+$ and $V_\beta6^+$ cells from SJL → (B10.S × AKR)F_1 with (B10.S × AKR)F_1 spleen cells or anti-V_β, the chimeric cells expressed IL-2 receptors (*21*). Addition of IL-2 partially restored the proliferation to anti-TCR stimulation, suggesting that at least one defect is in IL-2 production (Table 3). The failure to restore proliferation to control levels with the addition of IL-2 indicates

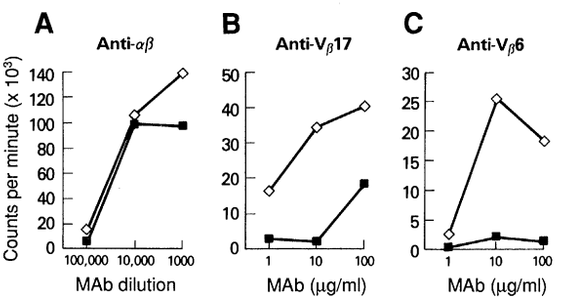

Fig. 4. P → F_1 chimeric T cells show poor responses to specific anti-V_β stimulation. $J11d^-$ thymocytes from SJL (◇) or SJL→F_1 (■) mice were incubated in 96-well U-bottomed microtiter wells previously coated with the indicated concentrations of a diluted anti-αβ-TCR ascites (**A**), purified anti-$V_\beta17$ (**B**), or anti-$V_\beta6$ (**C**). Responder cells were used at 1×10^5 cells per well, and chimeric cells were greater than 95% donor type. Cells were incubated for 72 hours, pulsed, and harvested as in Fig. 2. Data represent one of four similar experiments. Stimulation of CBA/J T cells, which lack the gene for $V_\beta17a$ and clonally delete cells bearing $V_\beta6$, resulted in proliferation values less than 10% that of normal SJL T cells.

Table 3. Nonresponsiveness to MAbs to the TCR in P → F_1 chimeras. Responders were $J11d^-$ thymocytes (1×10^5 cells per well) from SJL or SJL → (B10.S × AKR)F_1 chimeras. Chimeras were >95% donor type. Cells were incubated as in Fig. 4, except that suboptimal concentrations of anti-TCR were used and in the designated wells recombinant IL-2 (10 U/ml, Cetus) was added. The values (in counts per minute) represent the arithmetic mean (±SEM) of triplicate samples from one of two experiments; cells incubated in medium (±IL-2) were <1000 cpm.

Stimulation	Proliferation (cpm)			
	SJL		SJL → F_1	
	−IL-2	+IL-2	−IL-2	+IL-2
Anti-αβ	20,485 (±5,941)	43,711 (±4,589)	29,976 (±2,008)	49,442 (±3,807)
Anti-Vβ17a	16,663 (±666)	41,927 (±3,354)	2,428 (±511)	22,444 (±763)
Anti-Vβ6	5,885 (±1,295)	17,032 (±2,575)	1,469 (±15)	4,783 (±456)

that other defects exist in the tolerized cells, possibly involving TCR or IL-2 receptor signaling.

Our data suggest that the radioresistant host in P → F_1 chimeras is a poor inducer of clonal deletion, yet is capable of generating tolerance by a nondeletional mechanism. The tolerant state appears to be induced in the thymus since mature thymocytes and lymph node cells appear to manifest comparable levels of tolerance (*26*). Nondeletional mechanisms of tolerance to Mls and MHC can be generated in the periphery (*25, 27*). This study provides evidence that similar mechanisms may be responsible for intrathymic tolerance induction during T cell development. Although we cannot rule out the possibility that residual bone marrow–derived cells of host origin induce this tolerance, they would have to be extremely effective at inducing clonal anergy in a situation in which they are unable to promote efficient clonal deletion. Whereas the molecular parameters of this tolerant state and the means by which it is induced remain to be elucidated, these data demonstrate that the thymus is capable of inducing tolerance by at least two distinct mechanisms, clonal deletion and clonal anergy.

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15. V_β analysis of thymocytes is more reflective of thymic selection in chimeras. Donor lymphocyte turnover is more rapid and complete in the thymus than in the periphery. Turnover of nonlymphoid bone marrow–derived elements in the thymus, from host- to donor-type, can take up to 3 weeks (*4*); thus, T cells selected during this period by residual host cells will alter the peripheral T cell repertoire. Moreover, analysis of newly developing thymocytes minimizes the influence of environmental antigens or an extrathymic repertoire.
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17. We have substantiated this conclusion by also making F_1 → P chimeras [(CBA/Ca × B10.Q)F_1 → DBA/1], where the Mls-1^a antigen is contributed only by the H-2^q host, is unable to induce deletion of $V_\beta 6$. From these and the reverse P → F_1 chimeras, we demonstrated that deletion for $V_\beta 6$ occurs only when the bone marrow–derived elements bear an Mls-presenting haplotype (H-2^k), regardless of whether it is the host or donor that contributes the Mls.
18. T cells bearing $V_\beta 3$, which confers specificity for Mls-2^a, also were not deleted in normal SJL or in the SJL → (B10.S × AKR)F_1 chimera. The (B10.S × AKR)F_1 host is Mls-$2^{b\times b}$, however, since (SJL × AKR)F_1 mice delete $V_\beta 3$ (*21*), the normal SJL must express the Mls-2^a antigen, but H-2^s cannot present Mls-2^a for deletion. Thus, in the chimera the donor provides the Mls-2^a and the host expresses an appropriate MHC haplotype for presentation, but the chimera fails to mediate the relevant clonal deletion.
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Failure of T Cell Receptor V_β Negative Selection in an Athymic Environment

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The mature T cell receptor (TCR) repertoire is the result of selection events during T cell development. Previous assessment of TCR β-chain selection with serologic and molecular probes demonstrated both positive and negative selection. Although this work suggested a critical role for the thymus, no direct assessment has been made of the requirement for a thymus in TCR V_β selection. A comparison of TCR V_β expression in four different congenic pairs of normal and nu/nu (athymic) mice indicated that the normal V_β deletions associated with tolerance to self minor lymphocyte stimulating (Mls^c) antigens or to self major histocompatibility complex (MHC)–encoded $E_\alpha E_\beta$ products did not occur in most athymic mice. Thus, the thymus has a critical role in mediating self tolerance by negative selection.

MONOCLONAL ANTIBODIES (MAbs) specific for individual T cell receptor (TCR) V_β gene products (*1–7*) as well as molecular probes for gene expression (*8–11*) have been used to demonstrate negative selection of several V_β products during T cell repertoire generation. Strains that express MHC class II $E_\alpha E_\beta$ products delete peripheral T cells expressing $V_\beta 17a$ (*3*) or $V_\beta 11$ (*6*). Similarly, expression of Mls^a is associated with deletion of $V_\beta 6$ (*4*) and $V_\beta 8.1$ (*5*), and expression of Mls^c is associated with deletion of $V_\beta 3$ (*7, 9*). These deletions of V_β expression occur in mature thymocytes as well as in peripheral T cells (*3, 5, 7, 12*), suggesting involvement of the thymus in negative selection. We tested the requirement for a thymus in T cell repertoire selection by comparing TCR V_β expression in pairs of normal (thymus intact) and nu/nu congenic mice.

Although athymic nude mice are severely deficient in peripheral T cells, Thy-1^+ cells can be identified in these mice, especially as they become older (*13–16*). In the present studies, splenic T cells from 4- to 9-month-old nu/nu mice and euthymic congenic mice were analyzed for expression of $V_\beta 3$, $V_\beta 6$, $V_\beta 8$, and $V_\beta 11$ with appropriate MAbs. Nearly all (90 to 95%) Thy-1^+ cells from euthymic mice expressed the αβ TCR as determined by the MAb H57-597 (*17*), whereas 20 to 60% of Thy-1^+ cells from nude mice were $\alpha\beta^+$.

B10 normal and congenic B10 nu/nu spleen T cells had no deletions of $V_\beta 3$, $V_\beta 6$,

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