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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26. There was no evidence of peripheral T cell recirculation to the thymus in the chimeras. At early time points after reconstitution when $CD3^{Hi}$ thymocytes are <5% host type, the peripheral lymphoid organs are 20% to 80% host type.
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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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$V_\beta 8$, or $V_\beta 11$ in either population (Table 1), consistent with the fact that no deletions of these V_β's have been identified in B10 mice. In contrast to B10 mice, the strains BALB/c, C3H/HeN, and B10.D2 express $E_\alpha E_\beta$ products associated with deletion in $V_\beta 11$ expression (*6*). BALB/c and C3H also express Mls^c products associated with $V_\beta 3$ deletion (*7, 9*). No deletions in $V_\beta 6$ or $V_\beta 8$ are known to occur in these strains. Both $V_\beta 3$ (7.1% of $\alpha\beta^+$ cells) and $V_\beta 11$ (10.7% of $\alpha\beta^+$ cells) were detected in T cells from athymic BALB/c nu/nu mice, in contrast to the absence of $V_\beta 3$ (0.0%) or 11 (0.5%) in T cells from euthymic nu/+ littermates (Fig. 1). Thus, the TCR V_β deletions that occur in BALB/c mice as a result of expression of Mls^c and $E_\alpha E_\beta$ do not to occur in an athymic environment. This failure to delete $V_\beta 11$ was observed in seven of eight BALB/c nu/nu mice, and failure to delete $V_\beta 3$ was seen in seven of nine mice; euthymic BALB/c mice consistently (ten of ten mice) deleted both $V_\beta 3$ and $V_\beta 11$ (Table 1). Since T cell recognition of Mls^c and $E_\alpha E_\beta$ is mediated predominantly by $CD4^+$ cells, V_β expression was assessed in $CD4^+$ and $CD8^+$ cells. Some individual BALB/c nu/nu mice expressed high (undeleted) levels of $V_\beta 3$, $V_\beta 11$, or both in both of these T cell subpopulations (Fig. 2). In a comparison of C3H/HeN nude and normal Thy-1^+ T cells, $V_\beta 3$ and $V_\beta 11$ were deleted in euthymic but not in athymic T cells (Table 1). In similar experiments, euthymic B10.D2 T cells expressed $V_\beta 3$, $V_\beta 6$, and $V_\beta 8$ at frequencies comparable to those expressed by MHC congenic B10 T cells, but had fewer $V_\beta 11^+$ T cells (0.4% in Fig. 3), consistent with expression of $E_\alpha E_\beta$ by B10.D2 but not B10 mice (*6*). In contrast, 5.6% of B10.D2 nu/nu T cells were $V_\beta 11^+$ in the same experiment. Overall, eight of ten B10.D2 nu/nu mice did not delete $V_\beta 11$, expressing $V_\beta 11$ at a frequency equivalent to that observed in the $E_\alpha E_\beta$-negative strain B10 (Table 1). The examples of lower V_β expression (such as $V_\beta 6$ in BALB/c) in nude than in euthymic mice may represent a compensatory consequence of $V_\beta 3$ and $V_\beta 11$ nondeletion or may reflect a thymus-dependent component of positive selection for $V_\beta 6$ in this strain.

The reason that negative selection of $V_\beta 3$ and $V_\beta 11$ does not occur in athymic mice is not clear. It is possible that ligands responsible for V_β deletion are not expressed in nude mice. In the present studies, $E_\alpha E_\beta$ expression was equivalent on athymic and euthymic spleen cells; athymic and euthymic spleen cells were equally efficient as stimulators of Mls^c-specific T cells (*18*). However, the $E_\alpha E_\beta$ or Mls^c determinants required for T cell deletion may be distinct from those measured in other assays, and these tolerizing determinants may be absent in nude mice. It is possible that the thymus provides signals for T cell deletion that are not present in an athymic environment. A related possibility is that T cells are susceptible to negative selection only at a particular state in differentiation and that this state is generally not achieved in the absence of the thymus. It has been suggested that Mls- and $E_\alpha E_\beta$-specific deletions in the TCR V_β repertoire are dependent on CD4 expression and occur at the $CD4^+8^+$ stage (*12, 19, 20*). In agreement with previous reports (*21*), nude T cells characterized in the present study included single-positive $CD4^+$ and $CD8^+$ cells and double-negative $CD4^-8^-$ cells, but no detectable $CD4^+8^+$ cells (*18*). Thus, the lack of negative selection in nude mice might result from failure of $CD4^+8^+$ cells to

Table 1. T cell receptor V_β expression by athymic nude and euthymic T cells. Enriched T cells were prepared from euthymic and nu/nu athymic mice and were analyzed by two-color flow cytometry as described in Fig. 1. The number of T cells stained by antibody to a specific V_β was calculated as a percentage of total Thy-1.2^+ cells. The percentage of cells stained in negative control tubes was substracted from these values. The number of cells positive with each V_β-specific MAb was then expressed as a percentage of all TCR $\alpha\beta$-expressing cells as determined by staining with the MAb H57-597(*17*). Results are presented as the mean ± SE of results obtained for individual mice, with 7 to 12 mice of each strain examined.

Strain	V_β	Total TCR $\alpha\beta^+$ cells (%)	
		Euthymic	Athymic
C57BL/10	3	5.0 ± 0.8	8.9 ± 3.9
	6	8.7 ± 0.2	10.6 ± 2.9
	8	18.1 ± 0.4	10.6 ± 1.5
	11	4.6 ± 0.5	3.3 ± 1.2
BALB/c	3	0.2 ± 0.1	4.3 ± 1.3
	6	11.8 ± 0.1	3.9 ± 1.5
	8	24.3 ± 0.1	15.8 ± 3.9
	11	0.4 ± 0.1	6.0 ± 1.1
C3H/HeN	3	0.1 ± 0.1	3.9 ± 2.2
	6	15.8 ± 1.3	11.9 ± 2.3
	8	24.4 ± 2.5	15.5 ± 3.4
	11	0.9 ± 0.2	18.0 ± 6.6
B10.D2	3	4.4 ± 0.5	5.1 ± 2.2
	6	10.6 ± 0.3	9.6 ± 2.0
	8	20.2 ± 1.0	18.0 ± 3.3
	11	2.0 ± 0.6	5.0 ± 1.2

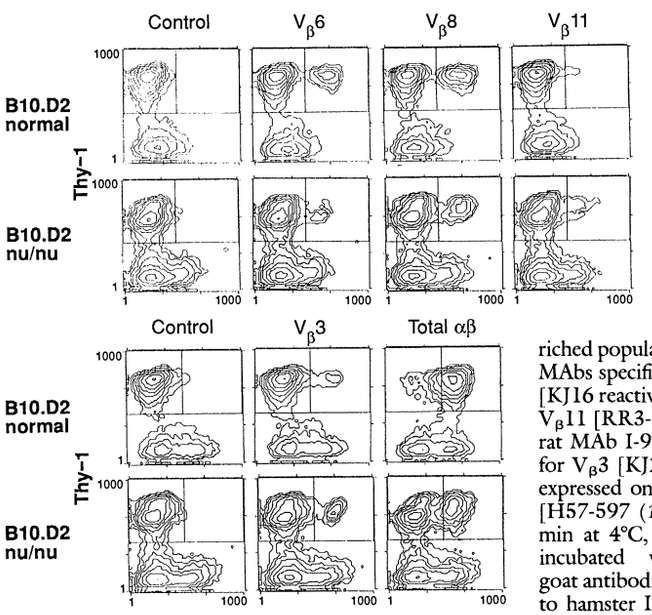

Fig. 1. TCR expression by T cells from BALB/c athymic (nu/nu) and normal heterozygous (nu/+) littermate mice. Spleen cells were enriched in T cells by depletion of surface immunoglobulin (Ig)-positive cells on rabbit antibody to mouse Ig plates. The enriched cells from nu/nu mice were 25 to 50% Thy-1^+ and from euthymic mice were 80 to 95% Thy-1^+. The T cell–enriched populations were incubated with rat MAbs specific for $V_\beta 6$ [RR4-7 (*32*)], $V_\beta 8$ [KJ16 reactive with $V_\beta 8.1$ and $V_\beta 8.2$ (*1*)], $V_\beta 11$ [RR3-15 (*6*)], the irrelevant control rat MAb I-9 (*33*), hamster MAbs specific for $V_\beta 3$ [KJ25 (7)], or for a determinant expressed on all TCR $\alpha\beta$-expressing cells [H57-597 (*17*)]. After incubation for 30 min at 4°C, cells were washed and then incubated with fluorescein-conjugated goat antibodies to rat Ig or goat antibodies to hamster Ig. Cells were then incubated with biotin-conjugated antibody to Thy-1.2 antigen (anti–Thy-1.2) for 30 min at 4°C in the presence of an excess of irrelevant rat Ig, washed, and incubated for 10 min with Texas red streptavidin. Two-color flow cytometric analysis was done on a modified Becton Dickinson dual-laser FACS II (*34*). Fluorescence intensities are expressed on a three-decade logarithmic scale. Minimum contour levels were set to correspond to approximately 0.01% of Thy-1^+ cells.

develop. Because some BALB/c nude mice have high proportions of $CD4^+$ cells expressing $V_\beta 3$, $V_\beta 11$, or both, nonexpression of CD4 does not completely account for the absence of negative selection. In the present study, T cells from nude mice consistently expressed a lower cell surface density of V_β than did euthymic T cells (Figs. 1, 2, and 3), consistent with previous reports (*21, 22*); this was true for potentially "selected" $V_\beta 3$ and $V_\beta 11$ and for "unselected" $V_\beta 6$ and $V_\beta 8$. In addition, fewer CD4 and CD8 molecules were expressed per cell in nude populations (Fig. 2). It is not clear whether these differences contributed to the lack of deletion. However, it has previously been observed in TCR transgenic mice that potentially self-reactive TCR can be expressed on peripheral T cells that have reduced or absent CD4 and CD8 expression (*19*). The frequency of expression of specific V_βs was also more variable among individual nude mice than among normal mice (Table 1), consistent with the oligoclonality of nude T cells (*23, 24*).

BALB/c, C3H/HeN, and B10.D2 nude mice that did not delete $V_\beta 3$ or $V_\beta 11$ showed no overt signs of autoreactive pathology that might be mediated by responses of $V_\beta 3^+$ or $V_\beta 11^+$ T cells to self antigens. T cells from athymic nude mice have a limited ability to respond to TCR-mediated stimulation (*15, 21, 25, 26*). Because the nondeleted T cells were not autoreactive in vivo, they may be developmentally immature. Alternatively, regulatory mechanisms may exist in vivo that suppress the potential reactivity of these T cells. In euthymic mice that have been rendered tolerant to alloantigen, it has been reported that T cells persist which express a V_β normally associated with reactivity to that alloantigen, but that these T cells are "anergic" (*27, 28*). It has not yet been determined whether the $V_\beta 3^+$ and $V_\beta 11^+$ cells expressed in nude mice in the present study respond normally to signals delivered through the TCR. However, it has recently been reported that $V_\beta 3^+$ T cells from BALB/c nude mice can be activated by *Staphylococcus* enterotoxin (*29*), a response that appears to be mediated through the αβ TCR (*30*), indicating that these T cells can respond to certain TCR signals.

Although T cell maturation and TCR expression occur in the absence of a thymus, the strong negative selection of TCRs that occurs in the maintenance of self tolerance to Mls^c and $E_\alpha E_\beta$ is largely dependent on the presence of a thymus. Extrathymic mechanisms for clonal deletion, if they exist, appear to be less effective than those mediated by the thymus, at least for T cells with potential reactivity to the Mls and MHC class II determinants studied. These findings are consistent with the report that neonatal thymectomy results in a TCR repertoire enriched in T cells expressing normally deleted $V_\beta 11$ (*31*). In this model, thymectomy resulted in organ-specific autoimmune disease, a consequence that has not yet been observed in congenitally athymic nude mice. The extrathymic pathway of T cell development that occurs in nude mice and in which negative selection appears to be deficient may coexist with a thymus-dependent pathway in normal mice; in such a setting the dominance of this latter pathway would obscure the existence of V_β nondeletion in extrathymically differentiated T cells.

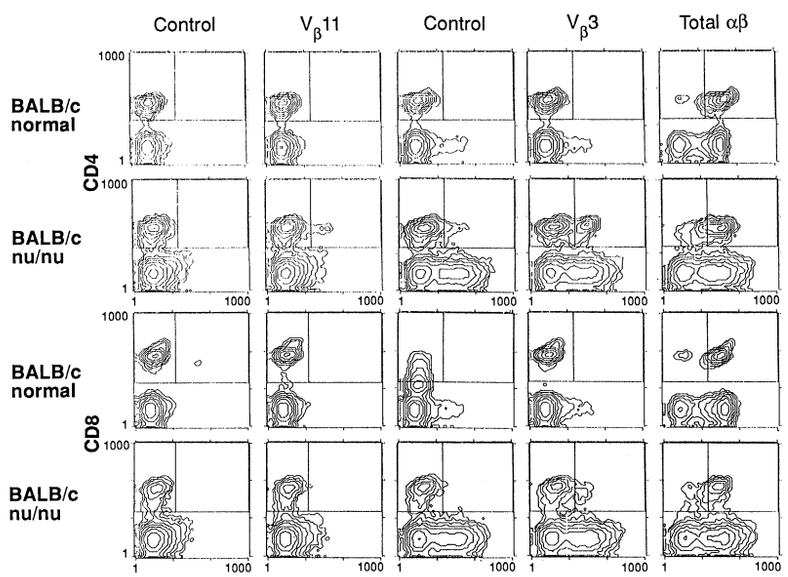

Fig. 2. TCR expression by $CD4^+$ and $CD8^+$ T cells from BALB/c athymic (nu/nu) and normal heterozygous (nu/+) littermate mice. Methods as described for Fig. 1, with the exception that biotin-conjugated antibody to CD4 or antibody to CD8 antigen was used in place of anti–Thy-1.2.

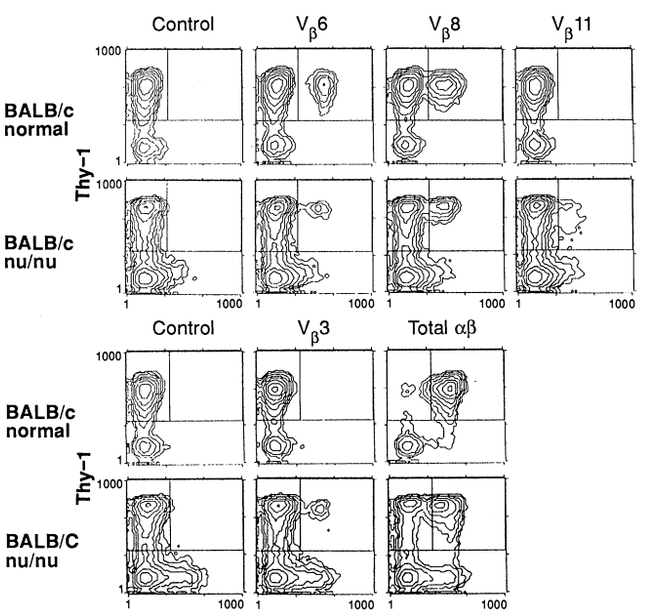

Fig. 3. TCR expression by T cells from B10.D2 normal and B10.D2 athymic (nu/nu) mice. Methods as described for Fig. 1.

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Thymic Requirement for Clonal Deletion During T Cell Development

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During T cell differentiation, self tolerance is established in part by the deletion of self-reactive T cells within the thymus (negative selection). The presence of T cell receptor (TCR)–αβ$^+$ T cells in older athymic (nu/nu) mice indicates that some T cells can also mature without thymic influence. Therefore, to determine whether the thymus is required for negative selection, TCR V$_\beta$ expression was compared in athymic nu/nu mice and their congenic normal littermates. T cells expressing V$_\beta$3 proteins are specific for minor lymphocyte stimulatory (Mls^c) determinants and are deleted intrathymically due to self tolerance in Mls^{c+} mouse strains. Here it is shown that V$_\beta$3$^+$ T cells are deleted in Mls^{c+} BALB/c nu/+ mice, but not in their BALB/c nu/nu littermates. Thus, the thymus is required for clonal deletion during T cell development.

THE TCR REPERTOIRE IS DETERMINED through positive and negative selection by self antigens in association with major histocompatibility complex (MHC) molecules (*1*). Studies with TCR-αβ transgenic mice (*2*) indicate that both processes occur within the thymus. Similar evidence has emerged from experiments with monoclonal antibodies (MAb) specific for individual murine TCR V$_\beta$ proteins (*3–6*). For example, certain TCR V$_\beta$ proteins, independent of the other components of the receptor, are specific for self antigens (for example, Mls) in association with particular class II MHC molecules (*3, 4, 7*). It has been demonstrated that T cells expressing these V$_\beta$ proteins are deleted within the thymus in mouse strains that express the self antigens.

Whereas most T cell development takes place intrathymically, some T cell maturation can also occur extrathymically (*8–10*). Older athymic nu/nu mice develop TCR-αβ–bearing T cells that are capable of both MHC-specific cytotoxic function and lymphokine production (*10*). To compare the TCR repertoire of inbred athymic (nu/nu) mice with that of euthymic (nu/+) control mice, we examined lectin-stimulated T cells from aged nu/nu BALB/c and B10 mice, as well as normal BALB/c and B10 mice, for the surface expression of various TCR V$_\beta$ proteins (Fig. 1).

BALB/c mice express Mls^c determinants associated with V$_\beta$3 deletion (*4, 7*), and E$_\alpha^d$E$_\beta^d$ Ia molecules. V$_\beta$11$^+$ T cells are deleted in mouse strains expressing E$_\alpha$E$_\beta$ gene products in association with currently undefined non-MHC self antigens (*11*). BALB/c mice express no self antigens associated with V$_\beta$8 deletion. Accordingly, the lectin-stimulated BALB/c T cell populations contained numerous V$_\beta$8$^+$ T cells, but almost no V$_\beta$3$^+$ or V$_\beta$11$^+$ cells (Fig. 1). In contrast, V$_\beta$3$^+$ and V$_\beta$11$^+$ as well as V$_\beta$8$^+$ T cells were found in the concanavalin A (Con A)–stimulated population (Fig. 1, A and B) from BALB/c nu/nu mice. No V$_\beta$ deletions have been found in B10 mice, and as shown in Fig. 1, V$_\beta$3$^+$, V$_\beta$8$^+$, and V$_\beta$11$^+$ T cells were observed in both the B10 and B10 nu/nu lectin-activated T cells.

Thus, BALB/c nu/nu mice, unlike their thymus-bearing littermates, fail to delete V$_\beta$3$^+$ and V$_\beta$11$^+$ T cells (*12*). However, the implications of this result for autoimmunity rest largely on demonstrating whether these T cells can respond to antigen stimulation. To address this important question, we examined the ability of nu/nu-derived T cells to respond specifically to stimulation with superantigenic staphylococcal enterotoxins (SE). SE stimulation appears to be mediated through the TCR because SE preferentially activate T cells expressing particular V$_\beta$ proteins (*6, 13, 14*). T cells from normal and athymic mice were activated with staphylococcal enterotoxin B (SEB), A (SEA), or E (SEE). SEB selectively stimulates V$_\beta$3$^+$ and V$_\beta$8$^+$ T cells (*6*), SEA stimulates V$_\beta$3$^+$ and V$_\beta$11$^+$ T cells (*6*), and SEE stimulates V$_\beta$11$^+$ T cells. T cells from nu/nu mice mounted a V$_\beta$-specific proliferative response when stimulated with SE. SEB-stimulated B10 and B10 nu/nu, as well as BALB/c nu/nu T cell populations were enriched for V$_\beta$3$^+$ and V$_\beta$8$^+$ cells (Fig. 2). However, even after SEB activation, the normal BALB/c population, which was highly enriched for V$_\beta$8$^+$ T cells, failed to express V$_\beta$3.

Analogous results were obtained on activation with SEA (Fig. 3A). In the experiment shown, V$_\beta$3$^+$ T cells constituted 36% of the SEA-stimulated BALB/c nu/nu TCR-αβ$^+$ T cells (*15*). SEA-activated normal BALB/c T cells were >98% TCR-αβ$^+$ (Fig. 3A), but very few were V$_\beta$3$^+$ (<2%). The distinction in V$_\beta$11 expression between BALB/c nu/nu and nu/+ T cells seen after lectin stimulation (Fig. 1) was no longer apparent after SE activation, such that many SEE-responsive normal V$_\beta$11$^+$ BALB/c T cells were found (Fig. 3B). However, this result is consistent with the fact that V$_\beta$11 deletion is often incomplete in E$_\alpha$E$_b$ mouse strains (*11*), and SEE activation presumably produced a dramatic expansion of the residual V$_\beta$11$^+$ BALB/c T cells. Nonetheless, V$_\beta$11$^+$ T cells from BALB/c nu/nu mice, like V$_\beta$3$^+$ cells, also respond specifically to SE.

In normal mice, clonal deletion appears to occur among immature CD4$^+$CD8$^+$ (double positive) cells (*5, 6*). TCR-αβ$^+$ CD4$^+$ or CD8$^+$ nu/nu T cells expressing V$_\beta$ that are deleted in their nu/+ counterparts might therefore develop directly from CD4$^-$CD8$^-$ precursors. Alternatively, they could arise from CD4$^+$CD8$^+$ cells, but the

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