Positive Selection Is Not Required for Thymic Maturation of Transgenic $\gamma\delta$ T Cells

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Summary

Previously published reports describing thymic differentiation in two TCR$\gamma\delta$ transgenic mouse models have suggested that $\gamma\delta$ T cells require MHC-mediated positive selection to reach full maturity. Recent studies indicate that recognition of antigen by mature $\gamma\delta$ T cells is not MHC restricted, raising the issue of why developing $\gamma\delta$ T cells would even require MHC-driven positive selection. Therefore, we have reinvestigated the requirements for development and selection in G8 $\gamma\delta$ T cell receptor (TCR) transgenic mice. Analyses of absolute cell numbers, phenotypic subsets, and functional competence of thymic and peripheral G8 $\gamma\delta$ T cells indicate that these cells can fully mature in class I MHC-deficient mice. Moreover, mixed bone marrow chimeras demonstrate that $\gamma\delta$ T cells of mutant B2-microglobulin ($\beta_{2m\lt}$) origin are partially deleted in the presence of H-2d-bearing thymocytes (previously believed to be the haplotype mediating positive selection). We conclude that there is no requirement for class I-like molecules for the maturation/development of these transgenic $\gamma\delta$ T cells and that the differences in thymocyte phenotype and number observed are, instead, attributable to effects of clonal deletion.

The major events in the intrathymic differentiation of $\alpha\beta$ T cells have been intensely investigated and well defined, while the maturation process for $\gamma\delta$ T cells is less well understood. Since the proportion of $\gamma\delta$ T cells in the thymus is <1% (1), studies on the development of these cells has been greatly aided by $\gamma\delta$ TCR transgenic mice. For our investigation, we have used the G8 mouse (2) that carries a transgene containing productively rearranged $\gamma$ and $\delta$ TCR genes derived from a T cell clone (3, 4) produced by immunization of BALB/c nu/nu mice with B10.BR spleen cells. The reactivity of the original T cell clone and of the $\gamma\delta$ T cells from the transgenic mice has been mapped to T22/T10 in the nonclassical (class Ib) region of the MHC (TL-region). The strongest reactivity was observed with H-2$^k$, intermediate with H-2$^b$, and no reactivity with H-2$^d$ antigen-presenting cells (APCs) (4).

Like classical MHC class I (class la) molecules, the putative class Ib ligand of the G8 TCR associates with $\beta_{2m\lt}$ (5); therefore, the absence of $\beta_{2m\lt}$ may influence the developmental fate of the transgenic $\gamma\delta$ T cells. Consistent with this idea were the reported phenotypic differences between the G8 $\beta_{2m\lt}$ and G8 $\beta_{2m\lt}$ thymuses that demonstrated a higher proportion of HSA~CD44~CD45R~B$^hi$ $\gamma\delta$ T cells in the class I$^-$ mice (6). Moreover, the G8 $\beta_{2m\lt}$ thymocytes did not proliferate to allogeneic stimulation, and these mice had very few, if any, peripheral lymphoid $\gamma\delta$ T cells (7). These data lead to the conclusion that $\gamma\delta$ T cells, like $\alpha\beta$ T cells, require MHC-dependent positive selection to complete their maturation. Similar conclusions were reached using another TCR$\gamma\delta$ transgenic strain, KN6, which has a similar pattern of recognition to G8 and was shown to react to T22, as well (8, 9). In contrast to these results, an investigation of $\gamma\delta$ development in non-transgenic $\beta_{2m\lt}$ mice indicated that many or most $\gamma\delta$ cells do not require class I or class I-like MHC for their development (10). These conflicting results could be explained if only a subset of $\gamma\delta$ T cells use MHC for positive selection.

In our efforts to investigate the cellular and molecular interactions involved in thymic $\gamma\delta$ T cell development, surprisingly, we found no requirement for $\beta_{2m\lt}$-associated molecules for full $\gamma\delta$ T cell maturation in G8 mice. Since the only other example of MHC requirement for $\gamma\delta$ development arises from a transgenic strain with very similar ligand reactivity to G8 (11), our findings raise the issue of whether any $\gamma\delta$ T cells require MHC-driven positive selection.

Materials and Methods

Mice. G8 transgenic mice (2) were backcrossed five times to B10.D2 mice and crossed two times to $\beta_{2m\lt}$ (H-2$^d$) mice (previously backcrossed five times to C57BL/10 (B10) mice) to gener-
ate TCRγδ transgenic, β2m" offspring (H-2b or H-2b). C57BL/6 mice were obtained from the National Cancer Institute (Frederick, MD) and B10.D2 mice from The Jackson Laboratory (Bar Harbor, ME). Mice were bred and housed in an NIAID Research Animal Facility according to AAALAC specifications. RAG-2" (H-2b) (12) and B2m" (13) mice (both backcrossed five times to C57BL/10) were bred and maintained on NIAID contract at Bioqual Inc. (Rockville, MD).

Monoclonal Antibodies And Flow Cytometry. For flow cytometric analyses, cells were stained according to standard protocols (14) using the following labeled antibodies: anti-TCRγδ-biotin (GL3), anti-TCRVγ2-biotin or -FITC (UC3-10A6), anti-TCRαβ-FITC or -PE (H57-597), anti-CD5(Ly-1)-biotin or -PE (IM7), anti-CD45RB-biotin or -PE (16A), anti-CD24/HSA-biotin or -PE (M1/69), anti-KL-FITC (SF1-1.1), all obtained from PharMingen (San Diego, CA); anti-TCRγδ-FITC (GL3), streptavidin-TC or -PE, from Caltag Labs. (San Francisco, CA); and avidin-APC (Molecular Probes, Eugene, OR). For cell sorting, cells were stained with anti-TCRγδ-biotin/APC- avidin, anti-HSA-PE, and anti-TCRαβ-FITC and sorted into γδ+αβ-HSA" and γδ+αβ-HSA" cell populations, respectively. To avoid FcR-mediated binding of antibodies, cells were pretreated with anti-Fc-receptor mono-

Figure 1. Two-color flow cytometric analyses reveal phenotypic differences between thymocytes from G8 β2m" (H-2b) and G8 β2m" mice. Freshly prepared thymocytes were stained with anti-TCRγδ mAb and the indicated antibodies. FACS® density plots are shown; data were collected using live gating for the TCRγδ" cells. (Data are plotted in two-color format to facilitate comparison with data from chimeras shown in Fig. 4.)

Figure 2. Thymocyte numbers in G8 β2m" (H-2b) and G8 β2m" mice. (Top) Total number of γδ thymocytes isolated from G8 β2m" (H-2b) and G8 β2m" mice, respectively. (Bottom) Number of HSA" γδ thymocytes per mouse derived from the product of the percentage of HSA" and γδ thymocytes and the total number of thymocytes. Each circle represents one mouse. Average values for each group are shown as columns: (top) 2.9 X 10^6 vs. 15.3 X 10^6; (bottom) 31.5 X 10^4 vs. 25.3 X 10^4.
clonal antibody (mAb), 24G2. Data were acquired and analyzed on a FACScan® using Cellquest, Lysys II, and PC-Lysys software, all from Becton-Dickinson (Mountain View, CA). Electronic cell sorting was performed on a FACStar® Plus (Becton-Dickinson).

Radiation Bone Marrow Chimeras. Bone marrow chimeras were made according to standard protocols (14). Briefly, NK cells of the recipients were depleted by injecting PK136 antibody (15) intraperitoneally the night before bone marrow transfer. Recipients were lethally irradiated (1,000 rads) 2-4 h before bone marrow transfer. Bone marrow suspensions were prepared from femurs and tibias of donors and were T cell depleted using anti-Thy-1.2 (J11) (16) and anti-Ly-1.2 (C3PO) (17) antibodies with low-tox rabbit complement (Cedarlane Labs, Westbury, NY). Recipients were injected with 2 × 10^7 donor bone marrow cells, intravenously. 5 wk after bone marrow transfer, the chimeric mice were killed and their thymuses were removed and analyzed.

Proliferation Assay. Responder cells were prepared from G8 TCRγδ transgenic thymocytes or lymph node cells (depleted of B cells using anti-mouse Ig-coated plates [14]) that were enriched for double negative (CD4^-CD8^-) cells using anti-CD8 (3-155) and anti-CD4 (KL-172) antibodies with low-tox rabbit complement (Cedarlane Labs.). Irradiated (3,000 rads) stimulator cells were prepared from C57BL/6 (H-2b) or B10.D2 (H-2d) spleen cells by T cell depletion (using anti-Thy-1.2 [J11] [16] antibody and rabbit complement), and 2 × 10^4 sorted responder cells were added to 3 × 10^5 stimulator cells per well in U-bottom 96-well plates. Proliferation was measured after 3 d of culture by [³H]thymidine incorporation (1 μCi/ml pulse for 18 h). Samples were harvested with a 96-well harvester (Brandel, Gaithersburg, MD), and [³H]thymidine incorporation was measured in a Betaplate counter (Pharmacia, Uppsala, Sweden). All values represent means of triplicate wells.

Results

To define the MHC requirements for γδ T cell development, we compared the phenotype and function of G8 TCRγδ + T cells of normal and MHC class I-deficient (β2m^-) mice. We analyzed thymocytes of these strains for surface expression of HSA, CD44, CD45, and CD5 (Fig. 1). In agreement with the previously published results (6), MHC class I^+ thymuses have a higher percentage of HSA^-CD44^-CD45RB^-CD5hi γδ thymocytes than those of class I^- mice. It is noteworthy that only class I^+ mice show a relative increase in γδ thymocytes bearing HSA^- and CD5hi, both considered to be markers of thymocyte maturation. Also of note is the enrichment in the class I^+ thymuses for cells bearing high levels of the activation markers CD44 (18) and CD45RB (19).

Surprising were our analyses of actual thymocyte numbers. Although there is a large variation in the number of γδ thymocytes, we find on average five to six times more of these cells in the β2m-deficient G8 mice (Fig. 2, top). This difference in cell numbers can be due to negative selection in the β2m^+ mice or to a failure of positive selection in the class I^-deficient mice, leading to an accumulation of immature (HSA^+) cells at the "pre-positive selection" stage. If the latter interpretation were correct, we would not expect to see any (or very few) HSA^- γδ T cells in the class I^- thymuses. The percentages of HSA^- thymocytes in Fig. 1 are deceiving, however, since, as determined by sampling a large pool of mice, the β2m^+ and β2m^- thymuses harbor comparable numbers of HSA^- γδ T cells (Fig. 2, bottom).

Figure 3. Three-color flow cytometric analyses show HSA^+ and HSA^- γδ T cells in the peripheral lymph nodes of G8 β2m^+ (H-2b) and G8 β2m^- mice. Freshly isolated peripheral lymph node cells were stained with anti-TCRγδ-FITC and anti-HSA-PE antibodies after pretreatment with anti-Fc-receptor antibody (24G2) to prevent FcR binding. FACSC® density plots are shown; data in c-d were collected live gated for TCRγδ^+ cells. Equivalent results are obtained with β2m^- mice of either the H-2d or H-2b haplotype.

Schweighoffer and Fowlkes
y8 T cell subpopulation in the lymph nodes of G8 mice has been resolved.

Although results with H-2 a APCs (not shown). The presence of liSA on y8 T cells is a marker of maturational stage, state of activation, or a lineage marker—a question that has not been resolved.

A distinct population of TCRγ8+TCRαβ− T cells is able to mature and reach the periphery of both the ß2m+ and ß2m− G8 mice (Fig. 3, a and b). These γ8 T cells are 85–90% CD4−8− and 10–15% CD4−8+. In addition, there is a small population of CD4+ T cells expressing both αβ and γδ TCR (Fig. 3, a and b). Although there has been no report of HSA+ γδ T cells in the peripheral lymphoid organs, we observe both a HSA+ and HSA− γδ T cell subpopulation in the lymph nodes of G8 mice (ß2m+ or ß2m−) (Fig. 3, c and d). The presence of HSA on a subset of peripheral γδ T cells raises the issue of whether HSA on γδ T cells is a marker of maturation stage, state of activation, or a lineage marker—a question that has not been resolved.

To demonstrate that the γδ T cells of the ß2m− mice are functional and bear the transgenic γδ TCR, TCRγδ+αβ− cells from G8 ß2m− thymuses and lymph nodes were sorted and assayed for specific alloreactivity (7). As shown in Table 1, regardless of whether γδ T cells developed in the presence or absence of ß2m and whether they are from the thymus or from lymph nodes, they all make specific responses to H-2×, but no response to H-27 or ß2m+ APCs. Although the HSA− γδ cells proliferate without exogenous cytokines, the HSA+ γδ T cell response is dependent on added IL-2.

Thus, in marked contrast to previous reports, where no γδ T cells appeared in the ß2m− peripheral lymphoid organs, these results demonstrate that γδ T cells of G8 mice can functionally mature and reach the periphery in the absence of ß2m. This argues against a developmental block due to the absence of class I and suggests, instead, that negative selection may be responsible for the reduced number of γδ thymocytes and the altered proportions of subsets in the H-2× mice.

To address this latter possibility, we constructed radiation bone marrow chimeras using stem cells from G8 ß2m− or G8 ß2m+ donors, or a mixture of these two bone marrows. Irradiated B10.D2 or ß2m+ mice served as recipients. These experiments allowed us to follow interactions between the ß2m+ and ß2m− G8 hematopoietic cells in the thymus, since the class I expression of the chimeric thymocytes could be used to identify the cells derived from each bone marrow donor. Table 2 shows that repopulation with either unmixed donor marrow (G8 ß2m−→ß2m+ or G8 ß2m+→ß2m+) results in similar γδ thymocyte numbers to those of intact animals (Fig. 2 a); that is, the G8 ß2m+ donors give rise to about five to six times more γδ thymocytes than do their class I− counterparts. More importantly, the lower number of G8 γδ thymocytes in the mixed chimeras, equivalent to those that received unmixed ß2m− marrow only (Table 2), reveals a dominant effect imposed by the ß2m+ hematopoietic cells, a property normally associated with negative selection.

Phenotypic analysis provided evidence that a subset of γδ T cells was the target of this deleterial effect (Fig. 4). β2m−-derived thymocytes are distinguished from β2m+-derived ones by staining with labeled anti-Kb class I antibody. The phenotype of the thymocytes in unmixed bone marrow chimeras (Fig. 4, a−c and g−i) corresponds to that of intact donors (Fig. 1) and is independent of the recipient's genotype, such that there is a higher percentage of the γδ thymocytes that are HSA+CD4−8− in the G8 β2m−→β2m+ chimeras (Fig. 4, g−i) than in the G8 β2m−→β2m+ chimeras (Fig. 4, a−c). In contrast, the G8 β2m+-derived thymocytes (Kb+) in the mixed bone marrow chimeras, which can interact with class I+ hematopoietic cells, were either absent or greatly diminished.
etic elements, acquire the phenotype of the G8 B2m\(^+\) (K\(^d^+\))-derived thymocytes; that is, they contain a higher percent of the HSA\(^b\),CD44\(^h\),CD45RB\(^h\) cells (Fig. 4, d-f). From these altered distributions, it appears that it is the immature HSA\(^b\),CD44\(^h\),CD45RB\(^h\) population that is the primary target of this negative selection. This deletion seems to be much less dramatic than the negative selection of \(\gamma\delta\) T cells originally described in H-2\(^b\) mice (2), in that the remaining cells are TCR\(\gamma\delta\)hi (as in the intact mice analyzed in Figs. 1 and 3), and they respond to stimulation with H-2\(^b\) APC. Similarly, there are well-documented examples of incomplete deletion in \(\alpha\beta\) T cell development, where coreceptor quantity or MHC levels, as well as quantity of peptide, can affect the degree of selection (20).

The question arises as to what cell types are mediating the deletion in the class I\(^+\) mice. Epithelial elements do not seem to play a role, since the class I haplotype expressed on the radiosensitive cells of the recipients does not seem to affect the developmental pattern in the bone marrow chimeras. Among the hematopoietic cells, the most obvious candidates are the macrophages, the dendritic cells, or the thymocytes themselves. To investigate this issue, we made radiation chimeras using bone marrow of G8 B2m\(^b\) mice mixed with RAG-2\(^b\) bone marrow. This latter strain was chosen because mice carrying mutations in both of their RAG-2 alleles lose the ability to generate T and B cells (12) but retain other hematopoietic cells. Fig. 5 shows that, unlike mixes made with nonmutant bone marrow, RAG-2\(^b\) hematopoietic cells do not mediate phenotypic changes in the \(\gamma\delta\) T cells derived from B2m\(^b\) marrow (Fig. 5, a-c vs. d-f).

**Figure 4.** Thymic phenotype of radiation chimeras. a–c and g–i show three-color flow cytometric analysis of thymocytes from chimeras made with unmixed bone marrow stained and analyzed similarly to the intact G8 B2m\(^b\) and G8 B2m\(^+\) (H-2\(^d\)) mice, respectively (shown in Fig. 1). d–f are analyses of mixed bone marrow chimeras. Cells derived from the G8 B2m\(^b\) vs. G8 B2m\(^+\) (H-2\(^b\)) bone marrow can be distinguished based on class I (K\(^d\)) expression. All plots were gated for \(\gamma\delta\) thymocytes. The numbers in parentheses represent percentages of each quadrant normalized for the percentages of B2m\(^b\)- or B2m\(^+\)-derived \(\gamma\delta\) thymocytes. FACS\(^\text{®}\) plots are representative of six experiments.

2037 Schweighoffer and Fowlkes
These latter results suggested that either thymocytes or thymic B cells could be inducing negative selection. To investigate this possibility, we bred G8 with RAG-2-deficient mice to produce G8 RAG-2−/− offspring selected to be homozygous for H-2d. The G8 RAG-2−/− mice contain about the same number of γδ thymocytes (3.1 ± 1.8 × 10⁶) as the G8 RAG-2+/+ animals, indicating that whatever the deleting element is in the H-2d mice, it must be present on the γδ cells themselves. Accordingly, chimeras made from G8 β2m−/− bone marrow mixed with G8 RAG-2−/− marrow induced deletion in the β2m−/−-derived (Kd) γδ thymocytes (Fig. 5, g−i). Although these findings demonstrate that γδ thymocytes are able to promote deletion, they do not exclude the αβ thymocytes or the thymic B cells as additional mediators of negative selection. We plan to assess the role of αβ thymocytes using H-2d TCR-β or TCR-α mutant mice since, in the absence of TCR-β, T cell maturation is blocked at the double negative (CD4−CD8−) stage, while in the TCR-α knockout mouse, thymocytes proceed to the double positive (CD4+CD8+) stage (21).

Discussion

TCR transgenic mice have been used extensively to study development and selection in αβ T cells. In contrast, requirements for γδ selection using such mice has been limited to two strains (G8 and K6). The ligand for the γδ TCR in both cases is class I, T22. It was somewhat perplexing that these transgenic γδ T cells failed to develop in β2m mutant mice whereas γδ development, in general, ap-

![Figure 5](image-url)
peared normal in nontransgenic class II\(^{\ast}\) and B2m mutant mice (10, 22). It was possible, nevertheless, that the transgenic \(\gamma\delta\) T cells represented a subclass of \(\gamma\delta\) T cells that required MHC for their development, implying that the requirements for \(\gamma\delta\) development were different and perhaps even more complex than those for the \(\alpha\beta\) lineage.

In an effort to compare and contrast the developmental requirements for the \(\alpha\beta\) and \(\gamma\delta\) T cell lineages, our intention was to identify selecting ligands and to determine whether thymic stromal elements also mediate \(\gamma\delta\) selection, as they do for \(\alpha\beta\) cells. In our studies of positive selection, we were surprised to find transgenic \(\gamma\delta\) T cells that were phenotypically and functionally mature in the thymus and periphery of class I-deficient mice (Figs. 1–3 and Table 1). Moreover, our preliminary data show that they are present in the lymph nodes and spleen of G8 scid B2m\(^{\circ}\) mice, as well, ruling out any role for endogenous receptors. Although we cannot pinpoint the cause of the discrepancies from the published data (6, 7), we suggest that whatever is responsible for the maturational block previously observed to be linked to B2m, this association is disrupted in the genetic combinations we used. The fact that both the class I\(^{\ast}\) G8 mice and the B2m\(^{\circ}\) mice used to generate G8 B2m\(^{\circ}\) offspring were five times backcrossed to the B10 strain raises the issue of whether background genes in the 129 (the strain used to produce the B2m mutation) are responsible for the earlier results and is an issue under investigation.

Not only did we find mature \(\gamma\delta\) T cells in our G8 B2m\(^{\circ}\) mice, but the higher (five to six times) number of \(\gamma\delta\) T cells in the B2m\(^{\circ}\) thymus suggested that there may even be some deletion in the presence of class I or class I-like molecules (Fig. 2). Evidence for this negative selection was provided by mixed radiation bone marrow chimera experiments, showing that, in the presence of class I\(^{\ast}\) hematopoietic cells, a portion of the immature class I-deficient \(\gamma\delta\) T cells disappeared (Fig. 4). Radiation chimeras made with mixtures of RAG-2\(^{\ast}\) and G8 RAG-2\(^{\ast}\) marrow indicated that the ligand mediating the deletion is present on the \(\gamma\delta\) cells themselves (Fig. 5). Whether \(\alpha\beta\) thymocytes also play a role in this process remains to be established.

The described ligand of the G8 clone appears to be encoded by the T22\(^{b}\) (23) and T10\(^{b}\) (24) genes of the MHC. The corresponding allele of T22\(^{b}\) in the H-2\(^{d}\) haplotype is structurally defective (5, 8); T10\(^{b}\), however, appears to be intact (8). In addition, T22 seems to be widely expressed (spleen, thymus, liver, kidney, etc.), while T10 is more restricted to spleen, thymus, and peritoneal exudate cells (8). Based on these data, T10 could be a potential candidate ligand causing the negative selection of transgenic \(\gamma\delta\) T cells that we observe in the B2m\(^{\circ}\) mice. Whatever the ligand is, our data suggest that it is not expressed on the epithelium or on the thymic APCs but rather on the thymocytes, as has been shown for other TL family members (25).

In an earlier publication (6), the enrichment for a subset of \(\gamma\delta\) thymocytes with a CD45RB\(^{b}\)Mel-14\(^{b}\) phenotype in G8 H-2\(^{d}\) mice that was not observed in B2m\(^{\circ}\) mice seemed to be consistent with the notion that activation markers could be expressed by cells undergoing positive selection. Since we show here that G8 \(\gamma\delta\) T cells in the H-2\(^{d}\) mouse are undergoing some negative selection, these activation markers could be restricted to cells engaged in clonal deletion. The fact that we do not detect proliferation of G8 \(\gamma\delta\) T cells from B2m\(^{\circ}\) mice in response to H-2\(^{d}\) APCs could be explained as it is for the \(\alpha\beta\) lineage; that is, that negative selection of thymocytes can be triggered by lower avidity interactions than those required for the activation of mature T cells (26, 27). The relative depletion of the HSA\(^{b}\) \(\gamma\delta\) cells in the H-2\(^{d}\) thymus, compared with those in B2m\(^{b}\) mice, indicates that the HSA\(^{b}\) cells are the target of this deletional mechanism. Thus, depletion of HSA\(^{b}\) \(\gamma\delta\) thymocytes would increase the relative proportion of HSA\(^{-}\) \(\gamma\delta\) cells, making it appear that more mature \(\gamma\delta\) thymocytes were present in the H-2\(^{d}\) than in the class I-deficient mice. The failure to see diminution of HSA\(^{-}\) \(\gamma\delta\) thymocytes in the thymus and in the periphery of the class I\(^{\ast}\) G8 mice either could be explained by the accumulation of mature cells that survive deletion or implies that the HSA\(^{+}\) cells are not precursors to the HSA\(^{-}\) cells.

Although MHC-restricted antigen responsiveness of \(\alpha\beta\) T cells is well documented, the rules for antigen recognition by \(\gamma\delta\) T cells remain elusive. \(\gamma\delta\) T cells have been reported to recognize a wide variety of antigens, including MHC class I (5, 28) and class II (29) molecules, mycobacterial peptides, heat shock proteins (30, 31), and small nonpeptide ligands (32). At the same time, recent studies investigating class I- and class II-specific \(\gamma\delta\) T cell clones have demonstrated that antigen processing and binding of peptides to the MHC molecules are not necessary for \(\gamma\delta\) T cell activation (23, 24). Taken together, these conflicting data suggest that the nature of \(\alpha\beta\) and \(\gamma\delta\) TCR recognition is quite distinct. Moreover, the requirements for T cell maturation in these two lineages seems to be different, in that nontransgenic B2m\(^{\circ}\) (10) and class II-deficient (22) mice seem to have normal \(\gamma\delta\) T cell populations. If \(\gamma\delta\) T cell antigen recognition is not MHC restricted, and if the purpose of thymic positive selection is to produce a large cohort of mature T cells that recognizes foreign antigens in the context of self-MHC, there should be no purpose for MHC-mediated positive selection in \(\gamma\delta\) development. This view is consistent with the data presented here. We have shown that the more mature, HSA\(^{b}\) \(\gamma\delta\) thymocytes exist in the B2m\(^{\circ}\) mice in numbers comparable to those in B2m\(^{\circ}\) thymuses and that functional transgenic cells are present in the periphery. We do not find any evidence for a developmental block in class I-deficient G8 \(\gamma\delta\) TCR transgenic mice. Indeed, we have demonstrated that class I-mediated positive selection is not necessary for \(\gamma\delta\) T cell maturation. Given the similarities in MHC recognition of G8 to KN6 (the only other TCR-\(\gamma\delta\) transgenic strain reported to require positive selection), we question whether MHC-driven positive selection is required in any \(\gamma\delta\) development. While we cannot exclude the possibility, of course, that B2m-independent ligands interact with \(\gamma\delta\) thymocytes at some point in their development and influence their fate, the existence and nature of such ligands have yet to be elucidated.
References


