Lifespan of γ/δ T Cells

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Summary

Information on the turnover and lifespan of murine γ/δ cells was obtained by administering the DNA precursor, bromodeoxyuridine (BrdU), in the drinking water and staining lymphoid cells for BrdU incorporation. For TCR-γ/δ (Vγ2) transgenic mice, nearly all γ/δ thymocytes became BrdU⁺ within 2 d and were released rapidly into the peripheral lymphoid tissues. These recent thymic emigrants (RTEs) underwent phenotypic maturation in the periphery for several days, but most of these cells died within 4 wk. In adult thymectomized (ATx) transgenic mice, only a small proportion of γ/δ cells survived as long-lived cells; most of these cells had a slow turnover and retained a naive phenotype. As in transgenic mice, the majority of RTEs generated in normal mice (C57BL/6) appeared to have a restricted lifespan as naive cells. However, in marked contrast to TCR transgenic mice, most of the γ/δ cells surviving in ATx normal mice had a rapid turnover and displayed an activated/memory phenotype, implying a chronic response to environmental antigens. Hence, in normal mice many γ/δ RTEs did not die but switched to memory cells.

Gamma/delta T cells comprise a minor subset of the T cells present in LN’s and spleen (1, 2). Although they clearly belong to the T cell lineage, γ/δ cells differ from conventional α/β T cells in many respects. In particular, γ/δ T cells recognize antigen in a manner fundamentally different from α/β T cells. Thus, most γ/δ T cells are not MHC restricted, and antigen recognition does not require the processing pathways involved in generating peptide-MHC complexes (2). Furthermore, γ/δ T cells have been shown to directly recognize nonpeptidic antigens, including phosphorylated nucleotides and prenyl pyrophosphate (3–6). Structurally, TCR-γ/δ may be more closely related to Ig molecules than to TCR-α/β, as suggested by primary sequence analysis (7). This differential recognition of antigen implies that γ/δ T cells may have a very different role than α/β cells in immune responses. In fact, it has been suggested that γ/δ cells may serve as a component of the innate immune system, as they have more functional similarities to macrophages and NK cells than to conventional T cells (8). A role for γ/δ cells in immune defense was suggested by their role in vivo expansion in response to certain bacterial, parasitic, and viral infections (9–16). In addition, antibody depletion of γ/δ cells and experiments with the TCR gene knockout mice indicated that γ/δ cells were important in the early response against intracellular bacteria (17–19). Nevertheless, the precise function of γ/δ cells remains unclear.

Understanding of the mechanistic basis of T cell function has been aided by the examination of lymphocyte lifespan. Thus, the in vivo lifespan of α/β T cells has been extensively studied and has yielded important information about the kinetics of thymocyte development, as well as the turnover of naive and memory T cells in the periphery (20–25). In contrast, no similar information is currently available regarding the lifespan of γ/δ T cells. Here, we have investigated the lifespan of γ/δ T cells in both TCR-γ/δ transgenic and normal mice by following bromodeoxyuridine (BrdU) incorporation in vivo. The results suggest that γ/δ T cells have a much more rapid turnover than do α/β T cells, both during development in the thymus and after export to the peripheral lymphoid tissues.

Materials and Methods

Mice. B6, DBA/2, and β-2-microglobulin-deficient (β2m−) mice were purchased from the rodent breeding colony at The Scripps Research Institute. G8 TCR-γ/δ transgenic mice (26) were provided by Dr. S. Hedrick (UCSD, San Diego, CA) and were backcrossed to the BALB/c background. Where indicated, mice were either thymectomized or sham operated at 5 wk of age. After the operations, the mice were given antibiotics in their drinking water and left for 4 wk before being used experimentally.

Abbreviations used in this paper: β2m, β-2-microglobulin; ATx, adult thymectomized; BrdU, bromodeoxyuridine; HSA, heat-stable antigen; RTE, recent thymic emigrants; STx, sham thymectomized.

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BrdU Treatment. Mice were given BrdU (Sigma Chemical Co., St. Louis, MO) in their drinking water at a concentration of 0.8 mg/ml. BrdU was dissolved in sterile water and was changed daily.

Adoptive Transfer. Vγ2+ T cells were purified from pooled LNs of TCR-γδ transgenic mice by treating cell suspensions with anti-heat-stable antigen (HSA) (11d), anti-CD8, and anti-CD4 plus complement (22). 2.5 × 10^6 cells were injected intravenously in PBS into B6 or β2m− mice that had been injected intraperitoneally with anti-NK-1.1 (PK136) 24 h earlier and irradiated (1,000 Gy) 4 h earlier. Recipients were then given BrdU for 4 d.

Antibodies and Cell Staining. Single cell suspensions were made from pooled LNs, thymus, or spleen and were surface stained using the following antibodies: Anti-Vγ2-PE (PharMingen, San Diego, CA); anti-CD4-PE (Collaborative Biomedical Products Co., New Bedford, MA); pan anti-TCR-γδ-PE (PharMingen); anti-CD45R B-biotin (PharMingen); anti-HSA-biotin (11d); anti-CD62L-biotin (Mel-14); and anti-CD44-biotin (IM7.8.1). Biotinylated antibodies were detected with streptavidin-RED613 (Becton Dickinson) and anti-BrdU-FITC (Becton Dickinson, Mountain View, CA). BrdU staining lymphoid cells for surface markers versus BrdU incorporation (anti-BrdU-FITC) occurred quite slowly in ATx mice and reached only 30% of cells by 21 d. In STx mice, by contrast, labeling of cells in LNs (Fig. 2 B) and spleen (data not shown) occurred quite slowly in ATx mice and reached only 30% of cells by 21 d. In STx mice, by contrast, labeling was rapid; thus, 45% of cells were labeled by day 7 and 70% by day 21.

Examining the surface markers on the BrdU-labeled cells revealed distinct differences between STx and ATx mice (Fig. 3). In ATx mice, the minor subsets of CD44hi, CD45R Bhi, and HSAhi cells had a rapid turnover. By contrast, the major subsets of naive CD44lo, CD45R Blo, and HSAlo cells had a very slow turn-over; for CD62L expression, turnover was slow for both CD62Lhi and CD62Llo cells. However, all subsets in STx mice had a more rapid turnover than in ATx mice.

There were two striking findings in STx mice. First,
BrdU labeling of HSA hi cells was very high (70%) as early as day 2; however, labeling of HSA lo cells was very low on day 2 but reached 40% by day 7. The simplest explanation for this finding is that the RTEs released from the thymus in STx mice were initially HSA hi but then switched very rapidly to HSA lo cells. Second, BrdU labeling of the major population of CD45RB int cells in STx mice was significantly slower than for HSA hi cells but reached high levels by day 7 (70%); labeling of CD45RB hi cells, by contrast, was almost undetectable on day 7 but then rose to 30% by day 21.

These data on STx mice suggest that upon exit from the thymus RTEs were initially CD45RB int and then gradually switched to CD45RB hi cells over a period of several days. However, this transition occurred much more slowly than did the switch of HSA hi cells to HSA lo cells, which would explain why STx mice contained far more CD45R B int cells than HSA hi cells (Fig. 1). It should be noted that BrdU labeling of CD45R B hi cells was appreciably slower in ATx mice (10% at day 21) than in STx mice (30% at day 21). This finding suggests that BrdU labeling of CD45R B hi cells in STx mice was largely a reflection of the labeling of precursor cells (CD45R B int cells) within the thymus rather than postthymic division in response to environmental antigens.

Phenotype of RTEs. Since BrdU labeling of naive phenotype cells on day 2 was conspicuous in STx mice but almost undetectable in ATx mice, it follows that the labeled cells found at day 2 in STx mice represented a relatively pure population of RTEs. As predicted from the above data, these 2 d-BrdU-labeled cells were nearly all CD45R B int rather than CD45R B hi, and most of the cells were HSA hi rather than HSA lo (Fig. 4). Typical of the naïve status of RTEs, the cells were also CD44 lo and CD62L hi.

Fate of RTEs. The data on the kinetics of BrdU labeling of CD45R B int and CD45R B hi cells in STx mice (Fig. 3) suggested that division of γ/δ RTEs in the extrathymic environment was minimal. However, whether RTEs differentiated into long-lived cells or died rapidly was unclear. To examine this question, STx mice were given BrdU continuously for 14 d and then transferred to normal drinking water for a further 28 d (Fig. 5). This pulse-chase approach showed that most RTEs had only a brief lifespan. Thus, after the BrdU pulse, the percentage of total BrdU + cells declined by ~70% during the 28-d chase period (Fig. 5).
genic mice were given BrdU for 2 d. Dot plots show representative staining of Vγ2+ cells in pooled LN cells.

Figure 4. Phenotype of γδ T lymphocytes in transgenic mice. STx (left) or ATx (right) TCR-γδ transgenic mice were given BrdU for 2 d. Dot plots show representative staining of Vγ2+ cells.

Figure 5. Pulse-chase BrdU labeling of Vγ2+ T cells in STx G8 TCR-γδ transgenic mice. Mice were given BrdU for 14 d ( ), or given BrdU for 14 d followed by normal drinking water for 28 d ( ). The percentage of BrdU+ cells in pooled LN cells is shown for (A) total Vγ2+ T cells; (B) Vγ2+ T cells expressing intermediate or high levels of CD45RB; (C) Vγ2+ T cells expressing low or high levels of HSA; and (D) Vγ2+ T cells expressing low, intermediate, or high levels of CD44.

With regard to surface markers, the disappearance of ~70% of BrdU+ γδ cells during the 28-d chase period applied equally to HSAlo, CD44lo, int, and hi, and CD62Llo and hi cells (Fig. 5, B–E, compare with Fig. 3). However, the loss of BrdU+ cells was much greater for the CD45RB int and HSAhi subsets (90–95%), consistent with rapid differentiation of these cells into CD45RBhi and HSAlo cells, respectively. For CD45RBint cells, it is notable that the disappearance of these cells was paralleled by a twofold increase in the proportion of BrdU+CD45RBhi cells (Fig. 5 B), thus providing direct support for the view that CD45RBhi cells arose from CD45RBint RTE precursors. Despite this finding, the data as a whole suggest that 70% of RTEs died within 1 mo of export.

γδ T lymphocyte kinetics. The observation that substantial numbers of BrdU-labeled cells appeared in the periphery of STx (but not ATx) mice within 2 d implied that the transit time of γδ cells through the thymus was very rapid. To examine this question directly, STx TCR-γδ transgenic mice were placed on BrdU and Vγ2+ cells in the thymus were analyzed (Fig. 6). By day 2, 90% of total Vγ2+ cells were BrdU+ (Fig. 6A). This rapid labeling also applied to cells having the phenotype of RTEs, i.e., to CD45RBint, HSAhi, CD44lo, and CD62Lhi cells (Fig. 6, B–E). These data indicated that Vγ2+ thymocytes had a very rapid transit and appeared to exit the thymus very soon after division. Interestingly, continuous administration of BrdU for up to 21 d failed to label 5–10% of total Vγ2+ cells in the thymus (Fig. 6A). These cells were predominantly CD45RBhi, HSAlo, CD44hi, and CD62Llo (Fig. 6, B–E). Two explanations could account for the presence of these cells. First, a fraction of the γδ cells generated in the thymus was unable to emigrate to the periphery and remained in situ for an indefinite period of time. Second, some mature γδ cells were able to recirculate from the periphery back to the thymus. Among αβ T cells, reentry into the thymus is restricted to activated cells (30). Whether the same restriction applies to γδ cells has not been studied, although it is of interest that the long-lived thymic γδ cells in the above experiment were CD44hi CD62Llo, a phenotype associated with activation/memory amongst αβ T cells. Therefore, it was important to examine the phenotypic changes that occurred after activation of mature γδ cells.

A division of γδ T cells. To directly determine the phenotype of activated γδ T cells, purified transgenic Vγ2+ LN cells were adoptively transferred into H-2d G8 mice into heavily irradiated H-2d (B6) or β2m− mice, and the recipients were given BrdU for 4 d (Fig. 7). Since the T22/T10 antigen is dependent upon β2m for its expression (31), one would not expect the Vγ2+ cells to be activated after transfer to the β2m− recipients. In fact, BrdU labeling of Vγ2+ cells after transfer into β2m− mice was very low. By contrast, after exposure to T22/T10 antigen for 4 d in normal B6 mice, virtually all of the injected Vγ2+ cells became BrdU+; moreover, 15-fold more Vγ2+ cells were recovered from B6 than from β2m− hosts, indicating a marked expansion of the cells to T22/T10 antigen in B6 mice. In terms of surface markers, most of the Vγ2+ cells in B6 hosts displayed the typical CD44hi phenotype of activated cells.

At the early time point examined, the Vγ2+ cells also showed partial downregulation of CD45RB and CD62L.
For obscure reasons, TCR expression on the activated Vγ2 cells in B6 hosts was appreciably lower than for the Vγ2 resting cells in B2m2 hosts. The above data indicate that exposure to specific antigen caused Vγ2 cells to divide and switch to an activated phenotype. In terms of CD44 and CD62L expression, these cells closely resembled the minor subset of activated/memory phenotype cells found in the thymus of G8 TCR transgenic mice (see above).

γδ Cells in Normal Mice

The finding that the vast majority of Vγ2 cells in G8 TCR transgenic mice had a naive phenotype implied that the reactivity of this monoclonal population for typical environmental antigens was minimal. Therefore, it was considered important to examine the turnover of γδ cells in normal mice. Data on the phenotype and turnover of Vγ2 and total γδ cells in ATx and STx normal B6 mice are discussed below; the cells studied were prepared from pooled LN s and spleen. Quite similar data were seen in DBA/2 mice (data not shown).

Phenotype in STx versus ATx Mice.

As mentioned earlier, the vast majority of Vγ2 cells in G8 TCR transgenic mice consisted of naïve phenotype cells, both in ATx and STx mice (Fig. 1). The situation in normal B6 mice was quite different (Fig. 8 A). In these mice a high proportion of Vγ2 cells in spleen and LN s displayed a CD44hi (memory) phenotype; CD44hi cells comprised ~45% of Vγ2 cells in STx mice and 85% in ATx mice. These findings contrasted sharply with the phenotype of αβ cells (Fig. 8 B). Thus, for CD4+ cells (which consist almost entirely of αβ cells), only a small proportion of these cells (25%) were CD44hi in ATx B6 mice; the majority of CD4+ cells were CD44int, the typical phenotype of naïve αβ cells.

Turnover in ATx Mice.

BrdU incorporation by Vγ2 cells in ATx mice was largely limited to memory phenotype CD44hi cells (Fig. 8 C). Since these cells comprised the bulk of Vγ2 cells (Fig. 8 A), the labeling of total Vγ2 cells in ATx mice was high and reached 70% by day 21 (Fig. 8 D). This high rate of labeling in ATx mice also applied to total γδ cells, i.e., to cells detected with a pan anti-γδ mAb (Fig. 8 E). For CD44 subsets, the rate of labeling of Vγ2 cells and αβ (CD4+) cells was quite similar, i.e., high for CD44hi (memory) cells and low for CD44lo/int (long-lived naïve) cells (Fig. 8 F). For other markers, BrdU labeling of CD45RB subsets of Vγ2 cells in ATx mice was much the same as for G8 TCR transgenic mice, i.e., high for CD45RBlo (memory) cells and low for CD45RBhi (naïve) cells (data not shown). As in G8 mice, labeling of CD62Lhi and CD62Llo subsets of Vγ2 cells was quite similar (data not shown).

Turnover in STx Mice.

In marked contrast to ATx mice, 

Figure 6. Turnover of Vγ2 thymocytes in G8 TCR-γδ transgenic mice. STx TCR-γδ transgenic mice were given BrdU for 2 ( ) or 21 ( ) d and Vγ2+ thymocytes were analyzed. (A) Total Vγ2+ thymocytes. (B) Vγ2+CD45RB thymocyte subsets. (C) Vγ2+HSA thymocyte subsets. (D) Vγ2+CD44 thymocyte subsets. (E) Vγ2+CD62L thymocyte subsets. Data represent mean values ± SD for two to three mice per point.

Figure 7. Phenotype of γδ cells activated in vivo. Vγ2+ T cells were purified from G8 TCR-γδ transgenic mice and injected into either B2m2 (upper) or B6 (lower) mice; recipients were treated with anti-NK-1.1 antibody and irradiated before adoptive transfer (see Materials and Methods). After injection of Vγ2+ T cells, recipients were given BrdU for 4 d. Dot plots show staining of spleen cells from representative mice.
the turnover of naive phenotype Vγ2−T cells in STx mice was rapid. Thus, BrdU labeling of CD44lo cells in STx mice reached 40% by day 7, compared with <5% for ATx mice (Fig. 8 C). It should be emphasized that naive phenotype Vγ2−T cells were a major population in STx mice. Thus, ~50% of Vγ2−T cells in STx were CD44lo/− cells, compared with only 15% in ATx mice (Fig. 8 A). The substantially higher frequency of naive phenotype Vγ2−T cells in STx mice compared to ATx mice also applied to total cell numbers. Thus, total numbers of CD44lo Vγ2−T cells in spleen plus LNs were about fourfold higher in STx than in ATx mice (data not shown). Thus, the implication is that, as in G8 TCR transgenic mice, most naive Vγ2 R T E s generated in B6 mice had a restricted lifespan.

Figure 8. T cell turnover and phenotype in B6 mice. (A) Percentage of Vγ2− T cells expressing low, intermediate, or high levels of CD44 in STx and ATx mice. (B) Proportion of Vγ2− and CD44− cells expressing low, intermediate, or high levels of CD44 in ATx mice. (C) BrdU labeling of total, CD44−/−, CD44−/− and CD44−/− Vγ2− T cells in STx and ATx B6 mice given BrdU for 7 d. (D) Kinetics of BrdU labeling of total Vγ2− T cells in ATx mice. (E) Comparison of BrdU labeling of Vγ2− T cells versus total TCR−γδ− T cells in ATx mice given BrdU for 14 d. (F) BrdU labeling of Vγ2− and CD44− T cells expressing different levels of CD44 in ATx mice given BrdU for 21 d. Data shown are for pooled LN cells and represent mean values ± SD for two to three mice per point.

4 days

Figure 9. Phenotype of Vγ2− R T E s in B6 mice. STx (left) or ATx (right) B6 mice were given BrdU for 4 d (upper) or 7 d (lower). Dot plots show representative staining of Vγ2− pooled LN cells. The percentage of cells falling within the boxed areas is indicated.

Phenotype of R T E s. The phenotype of R T E s in STx mice is shown in Fig. 9. When control ATx mice were placed on BrdU water for 7 d, most of the BrdU− cells detected at days 4 and 7 were CD44−/−CD45RBlo memory cells. Labeling of these memory phenotype cells was also apparent, although to a lesser extent, in STx mice. However, like G8 mice, STx B6 mice also contained discrete populations of BrdU−/− naive CD44−/− and CD45RBlo memory cells (Fig. 9). Conferring previous findings on the α/β R T E s generated in the normal thymus (24), the labeled Vγ2 R T E s found in STx B6 mice were predominantly BrdU−/− rather than BrdU−bright. As discussed elsewhere, the lower incorporation of BrdU by thymocytes compared to peripheral T cells presumably reflects enhanced cold target competition from DNA released by dying thymocytes (24).

Collectively, the above data indicate that, as in G8 TCR transgenic mice, the γδ cells found in the periphery of normal B6 mice comprised three broad categories of cells with different turnover rates. In STx B6 mice, ~50% of γδ cells in spleen and LNs were R T E s; these cells probably incorporated BrdU exclusively in the thymus and then differentiated into typical naive resting cells in the periphery. In ATx mice, a small proportion of R T E s survived for prolonged periods as long-lived naive cells with a very slow turnover. However, in marked contrast to G8 mice, the bulk of γδ cells in ATx B6 mice were memory phenotype cells with a rapid turnover. This finding suggests that, after export from the thymus, most R T E s in B6 mice did not die rapidly but instead differentiated into memory cells through contact with environmental antigens. The prominent conversion of naive R T E s to memory cells in B6 mice pre-
sumably accounts for the curious observation that the turnover of total Vγ2 cells in STx and ATx B6 mice was almost identical (Fig. 8C, left panel). Thus, numerically, the rapid turnover of memory cells in ATx mice happened to balance the rapid production of RTEs in STx mice. This was a clear contrast to G8 mice, for which the paucity of memory cells in STx mice led to much higher labeling of total Vγ2 cells in STx than in ATx mice (Fig. 8B).

Discussion

Like α/β T cells, the γ/δ T cells found in LN’s and spleen arise in the thymus and are subject to negative selection (1, 2, 8, 26, 32). Whether γ/δ cells undergo positive selection is less clear (31–37). In the case of the G8 TCR transgenic line (33) used here, and the closely-related KN6 line (34), the production of mature γ/δ cells was reported to be much lower in β2m-negative (β2m0) mice than in β2m+ mice, implying β2m-dependent positive selection. However, another group studying G8 mice found low but significant numbers of mature γ/δ cells in β2m0 mice and concluded that the γ/δ cells generated in β2m+ H-2d mice do not undergo positive selection but instead are subject to a covert form of negative selection (35). This possibility is difficult to reconcile with the finding that the production of G8 and KN6 γ/δ cells is substantially less in β2m0 than β2m+ H-2d mice and that the residual γ/δ cells in β2m0 mice have strong reactivity for H-2d but display no detectable reactivity for H-2a even in the presence of added IL-2 (35). Moreover, the data reported here show that the RTEs in β2m+ H-2d mice have a typical naive phenotype and do not display signs of activation.

This study shows that the kinetics of thymocyte development is much more rapid for γ/δ than α/β T cells. Studies on α/β thymocytes have shown that immature CD4+8– cells have a lifespan of ~3.5 d, whereas the turnover of the most mature thymocyte populations is much slower; these findings apply both to normal and TCR transgenic mice (20, 21). In contrast, we show here that, at least for TCR transgenic mice, the vast majority of γ/δ thymocytes including those having the phenotype of RTEs became BrdU+ within 2 d, indicating a very rapid rate of turnover. Hence, if γ/δ cells undergo positive selection (see above), this process must occur very rapidly. On the other hand, positive selection appears to be a time-limiting step in α/β T cell development. Positive selection of α/β thymocytes occurs soon after the transition of CD4–8– cells to cortical CD4+8– cells and induces a subset of these cells to differentiate into CD4+8– and CD4–8+ cells and migrate to the medulla (38). Although these steps in positive selection occur within several days, the subsequent export of mature α/β cells from the medulla into the extrathymic environment is slow and can take up to 1–2 wk (20). The reason for the prolonged residence of α/β thymocytes in the thymic medulla is unknown, although an obvious possibility is that additional selection steps are required before the cells are able to emigrate from the thymus. Whatever the explanation for the slow export of α/β cells, our data suggest that the release of γ/δ cells from the thymus occurs very rapidly. Thus, in G8 TCR transgenic mice, large numbers of labeled naïve γ/δ cells were apparent in peripheral lymphoid tissues of STx (but not ATx) mice after only 2 d on BrdU water. Hence, in contrast to α/β cells, γ/δ cells appear to be only dependent on the thymic microenvironment for a relatively brief period during their development.

It is of interest that γ/δ RTEs expressed a semimature phenotype. Thus, while RTEs resembled mature, naïve γ/δ T cells in expressing low levels of CD4 and high levels of CD62L, their phenotype was immature with regard to HSA and CD45RB expression, i.e., the cells were HSAhi and CD45RBint. These findings are consistent with the report that after intrathymic injection of FITC, most of the labeled γ/δ cells released from the thymus were CD44hi HSAhi cells (39). In this study, γ/δ RTEs matured from HSAhi, CD45RBint cells to HSAlo, CD45RBhi cells within 7 d after export from the thymus. It has been similarly reported that for CD4+ cells α/β RTEs are initially CD45RBint rather than CD45RBhi (24, 40). However, in contrast to γ/δ RTEs, only a small proportion of α/β RTEs express an immature HSAlo phenotype (40, 41). Therefore, a likely possibility is that those cells transiting most rapidly through the thymus, including some α/β and most γ/δ RTEs, exit as phenotypically immature cells. On this point, it is of interest that nearly all α/β RTEs in the rat emerge from the thymus as Thy-1+ CD45RC– cells and subsequently mature to a Thy-1+ CD45RC+ phenotype over a time span of ~7 d (42). An interesting question then is whether the immature phenotype of rat α/β RTEs also reflects rapid thymocyte kinetics. No information is currently available on this point.

In addition to rapid turnover in the thymus, most naïve γ/δ T cells were short-lived in LN’s and spleen in both TCR–γ/δ transgenic mice and normal mice. In TCR transgenic mice, the rapid turnover occurred in the absence of antigenic stimulation and, therefore, presumably reflected the death of naïve cells. Thus, a rapid rate of output of Vγ2+ cells from the thymus was balanced by a rapid loss of cells from the periphery. A high proportion of the cells appeared to die at a semimature CD45RBint stage. The loss of RTEs was not an artifact of the monoclonality of the Vγ2+ population in TCR transgenic mice, since rapid turnover of these cells was also observed in normal B6 mice. Although most RTEs were short-lived, a small proportion of these cells survived to become long-lived naïve cells. Thus, the majority of γ/δ cells in ATx transgenic mice, and a minority of cells in ATx B6 mice, displayed a typical naïve (CD44lo HSAlo CD45RBhi) phenotype and had a very slow turnover rate. Why these particular cells were selected for survival is unclear.

In nontransgenic B6 and also DBA/2 mice, it is of interest that most peripheral γ/δ cells acquired an activated/memory phenotype after thymectomy. Since these cells were rare in ATx TCR transgenic mice, the transition of naïve to memory phenotype cells in B6 mice presumably reflected an antigen-specific response to various environmental antigens. Unlike γ/δ cells found in epithelial tissues,
peripheral $\gamma\delta$ T cells express diverse TCRs and, therefore, are presumed to recognize a wide array of different antigens. However, the high frequency of memory phenotype $\gamma\delta$ cells in normal mice suggests that the $\gamma\delta$ T cell repertoire rapidly becomes biased towards recognition of frequently encountered antigens. As a consequence, with advancing age the $\gamma\delta$ T cell pool differs markedly from the $\alpha\beta$ population in having only a very small reservoir of naive cells. In this respect, in contrast to $\alpha\beta$ cells, the majority of $\alpha\beta$ (CD4+) cells in ATx mice display a naive phenotype (24).

Although the rapid turnover of memory phenotype $\gamma\delta$ cells in normal mice presumably reflected continuous or intermittent contact with antigen, some of the BrdU labeling may have represented bystander proliferation driven by cytokines, as has been observed for memory phenotype $\alpha\beta$ cells (43). This possibility is worth considering, since IL-12 cytokines, as has been observed for memory phenotype $\alpha\beta$ cells (45). These latter studies suggest that $\gamma\delta$ T cells may have some ability to mount a memory response to antigen. However, whether $\gamma\delta$ cells carry memory is still unclear. Most functional studies pointing to a role for $\gamma\delta$ cells in immune protection have focused on the primary response. Nevertheless, experiments with TCR- $\alpha\beta$ and $\gamma\delta$ knockout mice showed that $\gamma\delta$ cells do have a minor role in protection against a secondary challenge with Listeria monocytogenes (18). In addition, alloreactive $\gamma\delta$ TCR transgenic mice (on a SCID background) were shown to clear antigen-expressing cells more efficiently if the mice were primed with antigen 12 d before (45). These latter studies suggest that $\gamma\delta$ T cells may have some ability to mount a memory response to antigen. However, the phenotype of the cells responsible is unknown.

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