Immunity, Vol. 5, 217–228, September, 1996, Copyright ©1996 by Cell Press

MHC Class II Molecules Are Not Required for Survival of Newly Generated CD4$^+$ T Cells, but Affect Their Long-Term Life Span

Shunichi Takeda,*‡ Hans-Reimer Rodewald,* Hiroshi Arakawa,*§ Horst Bluethmann,† and Takeyuki Shimizu*
*Basel Institute for Immunology
Grenzacherstrasse 487
CH-4005 Basel
Switzerland
†F. Hoffmann–La Roche Ltd.
CH-4070 Basel
Switzerland

Summary

We grafted fetal thymi from wild-type mice into immunodeficient RAG-2$^{-/-}$ or class II$^{-/-}$RAG-2$^{-/-}$ (class II MHC$^-$) recipients and followed the fate of naive CD4$^+$ T cells derived from the grafts. In both types of recipients, newly generated CD4$^+$ T cells proliferated to the same extent in the periphery and rapidly filled the empty T cell compartment. However, CD4$^+$ T cells in class II$^-$ recipients gradually decreased in number over 6 months. These results show that interactions between the TCR and class II molecules are not required for newly generated CD4$^+$ T cells to survive and proliferate, but are necessary to maintain the size of the peripheral T cell pool for extended periods.

Introduction

Positive selection of T cells is a developmental process generating long-lived, mature CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$ cells from short-lived, immature CD4$^+$CD8$^+$ double-positive (DP) cells in the thymus. This process is initiated by weak interactions between the T cell antigen receptor (TCR) and major histocompatibility complex (MHC) molecules; interactions with class I or class II MHC molecules on thymic epithelium lead to the generation of single-positive (SP) CD8$^+$ or CD4$^+$ T cells, respectively (Fowlkes and Schweighoffer, 1995; Jameson et al., 1995; Kisielow and von Boehmer, 1995). Accordingly, only a few CD4$^+$ SP thymocytes and peripheral CD4$^+$ T cells are present in class II–deficient mice (Cosgrove et al., 1991; Grusby et al., 1991; Köntgen et al., 1993). There is evidence for the requirement of multiple MHC recognition events for positive selection in the thymus (Brandle et al., 1994; Chan et al., 1993; Pircher et al., 1994). However, it is not clear whether such weak interactions between the TCR and class II molecules are required for the survival of CD4$^+$ T cells in the periphery.

The thymus is crucial for formation of the T cell pool in early life. The degree of peptide/MHC diversity in the thymus seems to correspond with the size of the TCR repertoire of positively selected T cells (Ashton-Rickardt et al., 1994; Hogquist et al., 1993), although this is controversial (Schumacher and Ploegh, 1994). The thymus atrophies after puberty and plays little role in the maintenance of the T cell pool in secondary lymphoid organs (Sprent, 1993). In fact, the turnover of mature T cells is not detectably affected by adult thymectomy (Tough and Sprent, 1994), and a peripheral T cell pool is maintained in a self-renewing manner by cell division and cell death. Naive T cells, which have not encountered antigens, can survive for prolonged periods and are able to mount primary responses to new antigens in advanced age (Bruno et al., 1995). In addition, TCR Vα and Vβ usages of CD4$^+$ T cells do not change with age, suggesting that the TCR repertoire of CD4$^+$ T cells remains constant with age (Callahan et al., 1993). Since the total number of cells in lymphoid organs is relatively constant, dividing T cell clones would dilute out resting T cell clones. It is thus surprising that enormous diversity of the TCR repertoire is maintained for extended periods.

It is not clear whether T cells can divide in vivo without signaling through the TCR. The activation of mature CD4$^+$ T cells in vivo generally results from the binding of the TCR to specific immunogenic peptides embedded in MHC class II molecules, which are expressed mainly on professional antigen-presenting cells in the periphery. Similarly, it is believed that TCR-mediated signals are required for the proliferation of T cell clones in vitro. However, the following observations suggest that signals through the TCR do not play a major role in regulating the turnover of peripheral T cells. Mature T cells seem to be capable of surviving in vivo for extended periods in the absence of antigenic stimulations. This notion is supported by the observation that mature T cells transferred into immunodeficient *scid* mice survive indefinitely and that many maintain a virgin phenotype (Sprent et al., 1991). Furthermore, T cells seem to be activated to proliferate without prior antigenic stimulation. In fact, CD4$^+$ T cells in HIV patients may proliferate without prior antigenic stimulation, thus maintaining the peripheral CD4$^+$ T cell compartment for extended periods despite extensive cell death (Ho et al., 1995; Wei et al., 1995).

The present study addresses the following three questions: whether interactions between the TCR and class II molecules are still required for the survival of newly generated CD4$^+$ T cells; whether such interactions are required for the maintenance of the number and diversity of peripheral CD4$^+$ T cells for extended periods; and whether CD4$^+$ T cells can proliferate without receiving antigenic stimulation. To address these questions, we grafted fetal thymi derived from wild-type (class II$^+$) mice into class II$^-$ as well as class II$^+$ recipients and followed over time the fate of naive CD4$^+$ T cells derived from the grafted class II$^+$ thymi. The results show that newly generated CD4$^+$ T cells not only survived, but also actively proliferated in the absence of class II molecules in the periphery. Thus, weak interactions between the TCR and class II molecules are not required for the survival of newly generated CD4$^+$ T cells. The numbers

‡Present address: Department of Molecular Immunology, Kyoto University Medical School, Konoe Yoshida, Kyoto 606-01, Japan.
§Present address: Department of Biophysics, Kyoto University, Oiwake-cho Kitashirakawa, Kyoto 606-01, Japan.

of CD4$^+$ T cells in class II$^-$ recipients gradually decreased over 6 months, suggesting that interactions between the TCR and class II molecules affect the long-term survival of CD4$^+$ T cells and, consequently, the maintenance of the TCR repertoire of CD4$^+$ T cells.

Results

Engraftment of B6 Thymi in Class II$^+$ and Class II$^-$ Mice

We grafted day 15 fetal thymus from C57BL/6 (B6) mice into the kidney capsule of a class II$^+$ or class II$^-$ recipient. The thymic graft was expected to produce $\alpha\beta$ and $\gamma\delta$ T cells but no B cells (Frey et al., 1992). Since thymic epithelial cells, but not bone marrow–derived cells, are responsible for positive selection of CD4$^+$ T cells in the thymus (Lo and Sprent, 1986), thymic grafts in class II$^-$ mice were expected to produce CD4$^+$ T cells to the same extent as grafts in class II$^+$ recipients. With the engraftment of fetal thymi, we thus investigated the fate of recent thymic emigrants and presumably naive CD4$^+$ T cells in the periphery of class II$^-$ recipients.

We used the following four groups of H-2 identical (H-2^b) recipients: B6, class II$^{-/-}$B6 (Köntgen et al., 1993), immunodeficient recombination-activating gene-2 (RAG-2$^{-/-}$) (Shinkai et al., 1992), and RAG-2$^{-/-}$class II$^{-/-}$ mice. In B6 and class II$^{-/-}$B6 recipients, the engrafted thymi were expected first to produce T cells derived from donor-type precursors in the thymic grafts and, subsequently, from bone marrow–derived recipient-type precursors, which colonize the thymic grafts. On the other hand, in the RAG-2$^{-/-}$ recipients, which lack mature lymphocytes as a result of a defect in V(D)J recombination (Mombaerts et al., 1992; Shinkai et al., 1992), only donor-type T cells were expected to be produced for the first 4 weeks postgrafting (Frey et al., 1992). The fate of donor-type T cells can thus be followed without the subsequent input of host-derived T cells.

Since fetal thymi derived from B6 mice congenic for the Ly5.2 surface antigen were grafted into B6 or class II$^{-/-}$B6 animals, both of which were Ly5.1$^+$Ly5.2$^-$, donor-type T cells (Ly5.1$^-$Ly5.2$^+$) could be distinguished from recipient-type T cells (Ly5.1$^+$Ly5.2$^-$) by flow cytometric analysis. Figure 1A shows a representative flow cytometric analysis of peripheral blood from B6 and B6class II$^{-/-}$ recipients at 2 weeks postgrafting. Substantial numbers of Ly5.2$^+$ donor-type CD4$^+$ T cells were observed in both cases (Figure 1A, first and third columns), while at this timepoint no Ly5.1$^+$ recipient-type CD4$^+$ T cells were generated by the thymic grafts in B6class II$^{-/-}$ recipient mice (fourth column). The peripheral blood of recipients was analyzed every 1 or 2 weeks over a 7 month period, and the numbers of nucleated cells and percentages of CD4$^+$ and CD8$^+$ T cells among the nucleated cells were quantitated.

B6 Thymic Grafts Reconstituted Normal Numbers of Circulating CD4$^+$ T Cells in B6Class II$^{-/-}$ Mice

We analyzed the percentage of T cells among nucleated cells in the peripheral blood of B6 and B6class II$^{-/-}$ recipients over a 5 month period. Numbers of nucleated cells in the peripheral blood remained constant during the observation period and were comparable between the two groups of recipients. Percentages of CD4$^+$ versus CD8$^+$ T cells were approximately 20% versus 10% in B6 mice and 0.8% versus 24% in B6class II$^{-/-}$ mice. As shown in Figure 1B, at 3 weeks postgrafting percentages of donor-type T cells among nucleated cells reached the maximum level: 4.1% CD4$^+$ T cells and 1.3% CD8$^+$ T cells in reconstituted B6 mice, and 17% CD4$^+$ T cells and 2.9% CD8$^+$ T cells in reconstituted class II$^{-/-}$B6 mice (Figures 1B and 1D). The appearance of normal numbers of circulating CD4$^+$ T cells in the reconstituted B6class II$^{-/-}$ mice demonstrates that once CD4$^+$ T cells are positively selected in the thymus they no longer need engagement of the TCR with class II molecules to populate the periphery. Donor-type CD4$^+$ T cells expanded much more in B6class II$^{-/-}$ than in B6 recipients (Figure 1B). Presumably, newly generated CD4$^+$ T cells in B6class II$^{-/-}$ recipients proliferated in the periphery, while recent thymic emigrants in B6 recipients were not expected to proliferate in the periphery, as previously reported (Tough and Sprent, 1994).

CD4$^+$ T Cells Were Generated at the Same Level in RAG-2$^{-/-}$ and RAG-2$^{-/-}$Class II$^{-/-}$ Mice Grafted with B6 Fetal Thymi

The large population of donor-type CD4$^+$ T cells in B6class II$^{-/-}$ recipients (Figure 1B) was rapidly replaced by newly generated recipient-type CD4$^+$ T cells (Figure 1C). In both B6 and B6class II$^{-/-}$ recipients, most donor-type CD4$^+$ T cells disappeared at 10 weeks postgrafting; donor-type CD4$^+$ T cells among the nucleated cells were reduced to only 0.6% in B6 recipients and to 1.3% in class II$^{-/-}$B6 recipients (Figure 1B).

To investigate the fate of donor-type CD4$^+$ T cells for longer periods without interference by input of recipient-type T cells, we grafted day 15 fetal thymi of B6 mice into immunodeficient RAG-2$^{-/-}$ and RAG-2$^{-/-}$class II$^{-/-}$ mice. The peripheral blood of the recipients was analyzed every 1 or 2 weeks (Figure 2). The number of circulating CD4$^+$ T cells in both RAG-2$^{-/-}$class II$^{-/-}$ and RAG-2$^{-/-}$ recipients rose quickly and reached a normal level at 4 weeks postgrafting (Figure 2A). At 5 weeks postgrafting, the total number of CD4$^+$ T cells in secondary lymphoid organs was comparable in RAG-2$^{-/-}$ and RAG-2$^{-/-}$class II$^{-/-}$ recipients; numbers of CD4$^+$ T cells reached approximately 2.5×10^6 in the lymph nodes (LNs) and 2×10^6 in the spleen of RAG-2$^{-/-}$ recipients, and 2×10^6 in the LNs and 2.5×10^6 in the spleen of RAG-2$^{-/-}$class II$^{-/-}$ recipients (Figure 3). These observations show that there were no significant differences between RAG-2$^{-/-}$ and RAG-2$^{-/-}$class II$^{-/-}$ recipients with regard to the number of CD4$^+$ T cells populating the peripheral blood, LNs, and spleen. Hence, interactions between the TCR and class II MHC molecules did not affect the formation of the peripheral CD4$^+$ T cell pool.

Newly Generated CD4$^+$ T Cells Can Proliferate Even in the Absence of Class II MHC Molecules in the Periphery

To analyze the turnover of the T cells, we labeled dividing cells in RAG-2$^{-/-}$ and RAG-2$^{-/-}$class II$^{-/-}$ recipients with

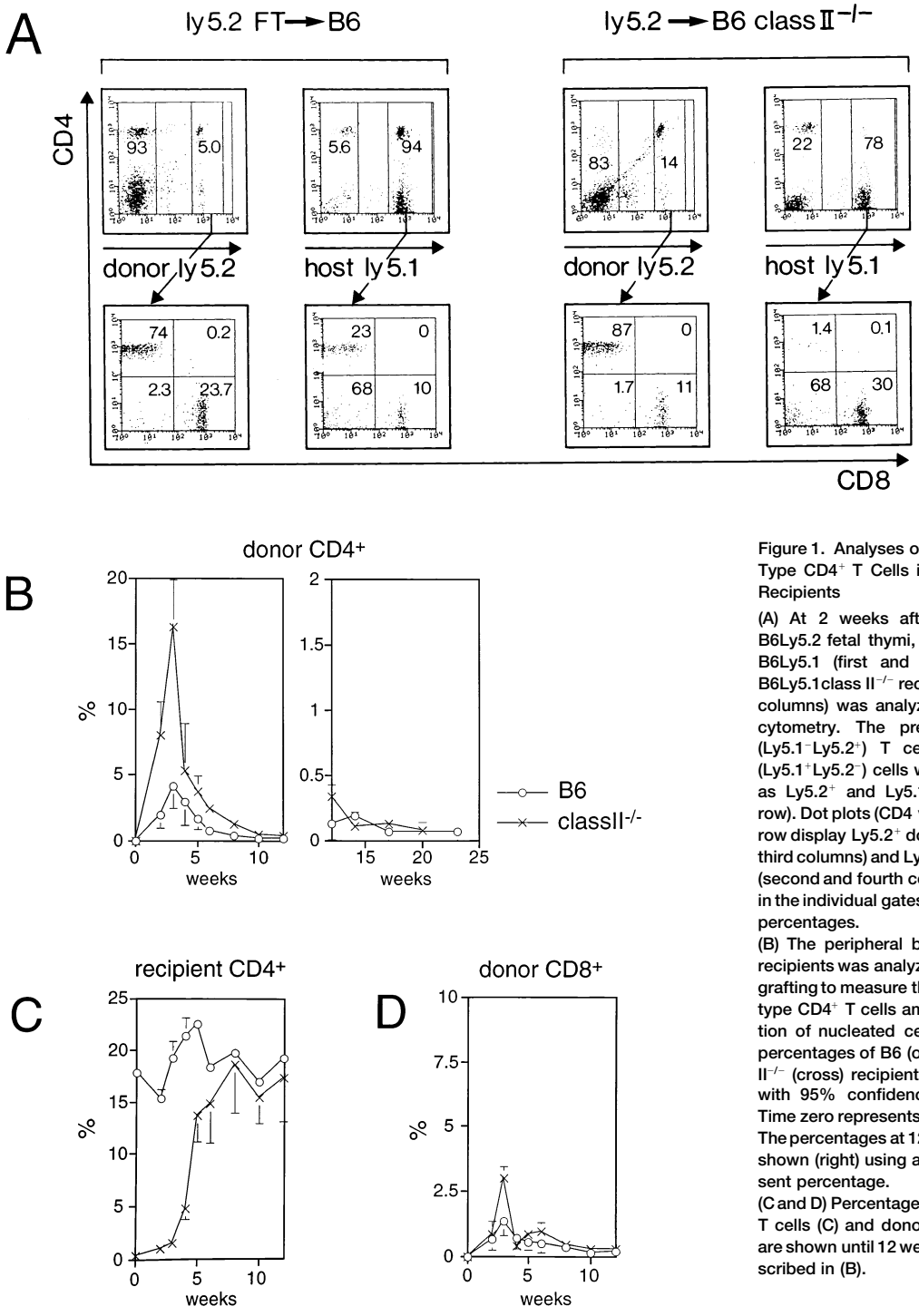

Figure 1. Analyses of Donor- and Recipient-Type CD4$^+$ T Cells in B6 and B6Class II$^{-/-}$ Recipients

(A) At 2 weeks after the engraftment of B6Ly5.2 fetal thymi, the peripheral blood of B6Ly5.1 (first and second columns) and B6Ly5.1class II$^{-/-}$ recipients (third and fourth columns) was analyzed by three-color flow cytometry. The presence of donor-type (Ly5.1$^-$Ly5.2$^+$) T cells and recipient-type (Ly5.1$^+$Ly5.2$^-$) cells was positively identified as Ly5.2$^+$ and Ly5.1$^+$, respectively (upper row). Dot plots (CD4 versus CD8) in the lower row display Ly5.2$^+$ donor-type cells (first and third columns) and Ly5.1$^+$ recipient-type cells (second and fourth columns). Numbers given in the individual gates and quadrants indicate percentages.

(B) The peripheral blood of two groups of recipients was analyzed over 23 weeks postgrafting to measure the percentage of donor-type CD4$^+$ T cells among the whole population of nucleated cells. Average values for percentages of B6 (open circle) and B6class II$^{-/-}$ (cross) recipients (n = 9–13) are shown with 95% confidence limits (vertical bar). Time zero represents the day of engraftment. The percentages at 12 weeks postgrafting are shown (right) using a smaller scale to represent percentage.

(C and D) Percentages of recipient-type CD4$^+$ T cells (C) and donor-type CD8$^+$ T cells (D) are shown until 12 weeks postgrafting, as described in (B).

5-bromo-2-deoxyuridine (BrdU), as previously described (Tough and Sprent, 1994). Continuous BrdU labeling was started at 5, 10, and 24 weeks postgrafting, and the percentage of BrdU-labeled CD4$^+$ and CD8$^+$ T cells in spleen and LNs was examined 5 days later. There was no reduction in the number of lymphocytes during the labeling experiments, indicating no toxic effects of BrdU on lymphocytes.

The emigration of T cells from the grafts in RAG-2$^{-/-}$ and RAG-2$^{-/-}$class II$^{-/-}$ recipients virtually ceased at 4 weeks postgrafting. In fact, in the peripheral blood of B6 and B6class II$^{-/-}$ recipients at 4 weeks postgrafting, the number of donor-type CD4$^+$ T cells decreased, while those of recipient-type CD4$^+$ T cells increased (Figures 1B and 1C). In agreement with this observation, precursor cells have been reported to need 3–4 weeks to complete their development in the thymus and to emigrate to the periphery (Shortman et al., 1990). Thus, after 4

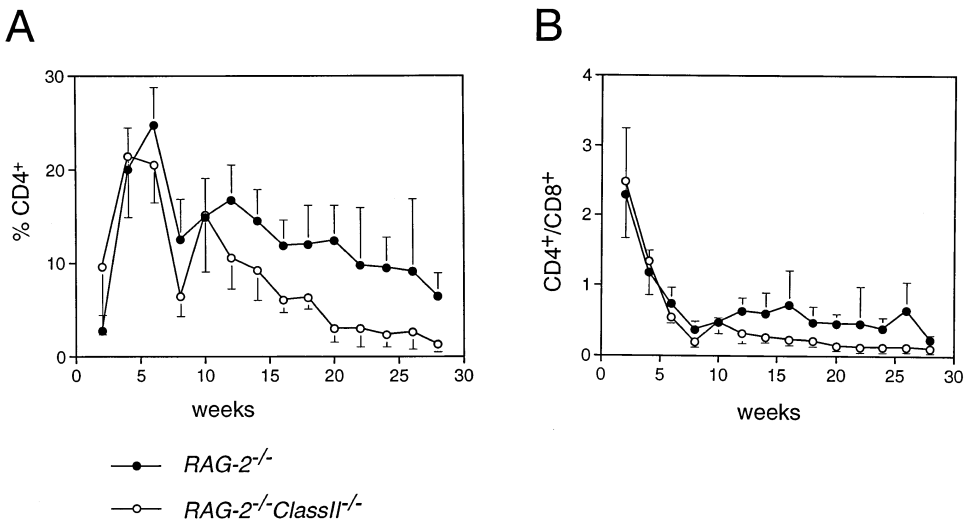

Figure 2. Analyses of Engrafted Thymus-Derived T Cells in RAG-2$^{-/-}$ and RAG-2$^{-/-}$Class II$^{-/-}$ Recipients

(A) The peripheral blood of two groups of recipients was analyzed over 28 weeks postgrafting to measure the percentage of CD4$^+$ T cells among the whole population of nucleated cells. Average values for RAG-2$^{-/-}$ (closed circle) and RAG-2$^{-/-}$class II$^{-/-}$ (open circle) recipients (n = 5–18) are shown with 95% confidence limits (vertical bar). Time zero represents the day of engraftment.

(B) Ratios of CD4$^+$ T cells/CD8$^+$ T cells in the peripheral blood are shown as described in (A).

weeks postgrafting, BrdU was expected to be incorporated mostly by peripheral T cells, but not by donor-type thymocytes.

At 5 weeks postgrafting, approximately 90% of the CD4$^+$ and CD8$^+$ T cells in RAG-2$^{-/-}$ and RAG-2$^{-/-}$class II$^{-/-}$ recipients were found to be labeled with BrdU (Figure 4A), indicating that these cells had divided for a 5 day period. To confirm that the labeled T cells had proliferated in the periphery, we also performed pulse–chase experiments at 5 weeks postgrafting; we gave some recipients water containing BrdU for 5 days (pulse label) and subsequently normal water for 2 weeks to examine the rate of decay of BrdU in labeled cells (chase), as described previously (Tough and Sprent, 1994). During the chase period, there was a marked switch from BrdU^{high} to BrdU^{low} CD4$^+$ cells, suggesting that the BrdU concentration of peripheral BrdU^{high} CD4$^+$ T cells had been diluted through extensive cell cycling (Figure 4A). These observations revealed that the vast majority of newly generated CD4$^+$ T cells were actively

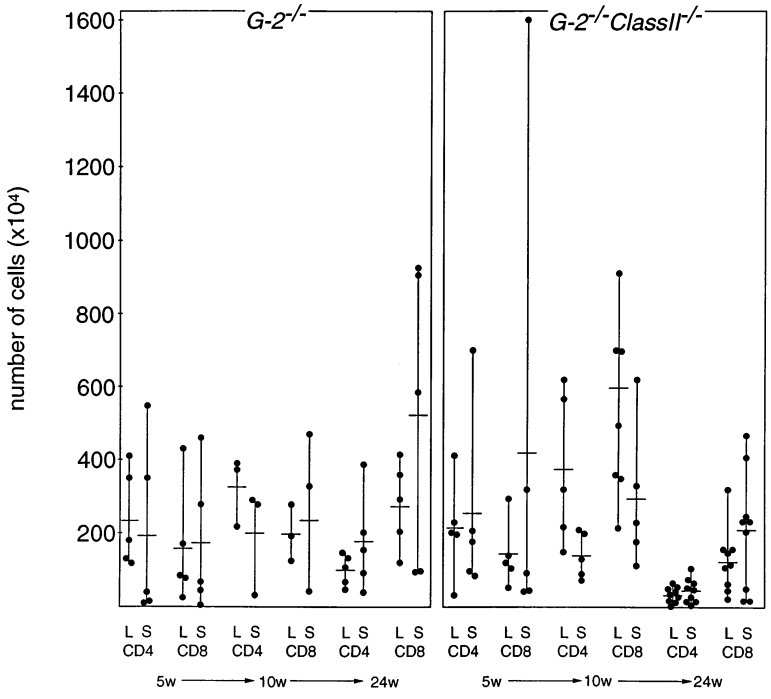

Figure 3. The Total Number of CD4$^+$ and CD8$^+$ T Cells in Lymph Nodes and Spleen of RAG-2$^{-/-}$ and RAG-2$^{-/-}$Class II$^{-/-}$ Recipients

Each dot represents the analysis of an individual recipient mouse, and the crosshatch on each line represents a mean median. Recipients were sacrificed at 5, 10, and 24–28 weeks postgrafting, and spleen and LNs were isolated. The number of nucleated cells was counted, and the percentage of CD4$^+$ and CD8$^+$ T cells among the nucleated cells was assessed by flow cytometric analysis. L and S represent LNs and spleen, respectively.

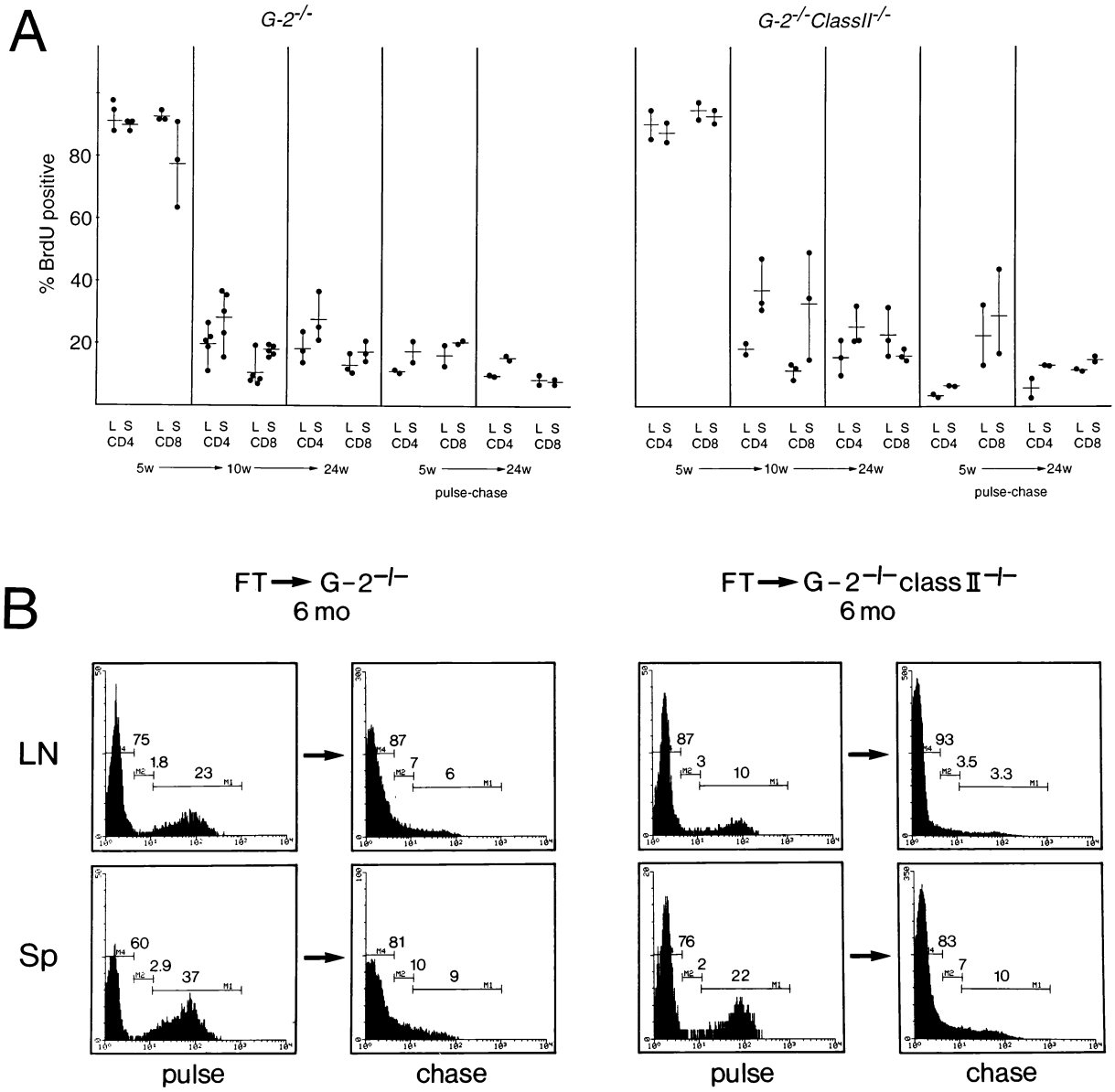

Figure 4. Percentages of BrdU-Labeled CD4$^+$ and CD8$^+$ T Cells in RAG-2$^{-/-}$ and RAG-2$^{-/-}$Class II$^{-/-}$ Recipients

(A) At 5, 10, and 24 weeks postgrafting, RAG-2$^{-/-}$ and RAG-2$^{-/-}$class II$^{-/-}$ recipients were given BrdU for 5 days through drinking water. At 5 and 24 weeks postgrafting, some recipients were used for the pulse (5 days)–chase (2 weeks) experiment. Sorter-purified CD4$^+$ and CD8$^+$ T cells from lymph nodes (L) or spleen (S) were analyzed to measure the percentage of BrdU-labeled cells. Each dot represents the analysis of an individual mouse, and horizontal bars indicate the mean value.

(B) Representative histograms show amounts of BrdU incorporated by CD4$^+$ T cells from LNs and spleen (Sp). At 6 months postgrafting, RAG-2$^{-/-}$ recipients (first and second columns) and RAG-2$^{-/-}$class II$^{-/-}$ recipients (third and fourth columns) were pulse labeled for 5 days (first and third columns) or used for pulse–chase experiments (second and fourth columns). Sorter-purified CD4$^+$ T cells were fixed, stained with fluorescent-conjugated anti-BrdU antibody, and analyzed by flow cytometry. Numbers given indicate the percentage of cells over the population range indicated by each horizontal bar.

cycling in the periphery of RAG-2$^{-/-}$class II$^{-/-}$ as well as RAG-2$^{-/-}$ recipients. Circulating donor- and recipient-type CD4$^+$ T cells in B6class II$^{-/-}$ recipients rose with the same kinetics as CD4$^+$ T cells in RAG-2$^{-/-}$ recipients, suggesting that newly generated CD4$^+$ T cells in B6class II$^{-/-}$ recipients also proliferated in the periphery. These observations show that recent thymic emigrants can proliferate in the absence of TCR occupancy, when the number of peripheral T cells is not at a normal level.

In Class II$^-$ Recipients, the Number of CD4$^+$ T Cells Dropped Steadily over Prolonged Periods, although Many CD4$^+$ T Cells Were Actively Proliferating

In RAG-2$^{-/-}$class II$^{-/-}$ recipients from 10 to 24 weeks postgrafting the proportion of CD4$^+$ T cells in the peripheral blood dropped significantly, from 15% to 1.4% (Figure 2A), and the numbers decreased from 3.8×10^6 to 3.2×10^5 in LNs and from 1.4×10^6 to 4.0×10^5 in the

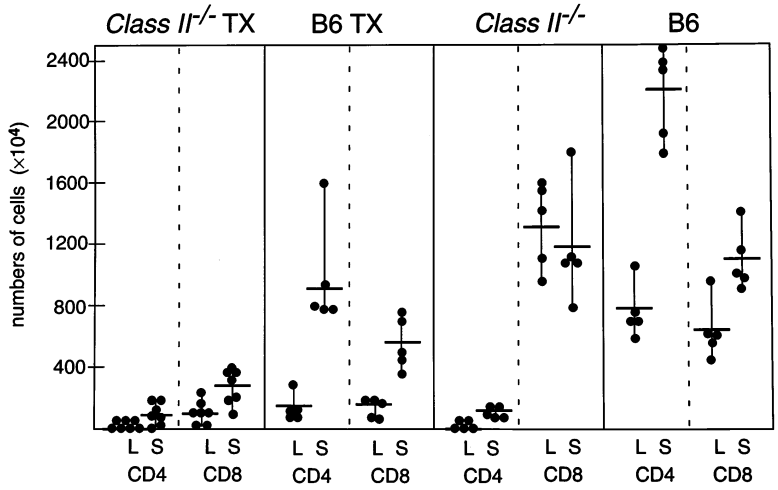

Figure 5. The Total Number of CD4+ and CD8+ T Cells in Lymph Nodes and Spleen of B6 and Class II−/−B6 at 6 Months after Thymectomy

Each dot represents the analysis of an individual recipient mouse, and the crosshatch on each line represents a mean median. Mice were sacrificed at 6 months after thymectomy (TX), and spleen and LNs were isolated. Age-matched unmanipulated B6 and class II−/−B6 mice were also analyzed. The number of nucleated cells was counted, and the percentage of CD4+ and CD8+ T cells among the nucleated cells was assessed by flow cytometric analysis. L and S represent LNs and spleen, respectively.

spleen (Figure 3). During the same period, the proportion and number of CD4+ T cells in RAG-2−/− recipients dropped to a lesser extent: from 15% to 9.6% in the peripheral blood (Figure 2A), from 3.3 × 10⁶ to 1.0 × 10⁶ in LNs, and from 2.0 × 10⁶ to 1.8 × 10⁶ in the spleen (Figure 3). Hence, in the absence of class II molecules, the number of CD4+ T cells decreased more rapidly.

At 10 and 24 weeks postgrafting, the percentage of BrdU-labeled CD4+ T cells ranged between 20% and 40% for both groups of recipients (Figure 4). Pulse–chase experiments were also performed at 24 weeks postgrafting. By the pulse labeling protocol, 10%–20% of the CD4+ T cells in the LNs and 25%–40% in the spleen incorporated BrdU in both groups of recipients (Figure 4). Subsequently, there was a marked switch from BrdU^{high} to BrdU^{low} cells during the chase period, indicating the rapid turnover of CD4+ T cells (with dilution of label; Figure 4B). In summary, the number of CD4+ T cells was gradually declining in RAG-2−/−class II−/− recipients, although a substantial fraction of these T cells were cycling.

CD4+ T Cells Contribute to the Maintenance of the CD8+ T Cell Compartment

In RAG-2−/−class II−/− recipients, the number of CD8+ T cells declined with the same kinetics as for CD4+ T cells from 10 to 24 weeks postgrafting in secondary lymphoid organs (Figure 3), although the expression of class I MHC molecules in these recipients was normal. Similarly, 6 months after thymectomy, the number of peripheral CD8+ T cells was smaller in class II−/−B6 mice than in B6 mice, although unmanipulated class II−/−B6 mice contained more CD8+ T cells than B6 mice (Figure 5). These observations imply that the presence of activated CD4+ T cells affects the life span of CD8+ T cells. These results are in agreement with previous observations (Bruno et al., 1995; Kirberg et al., 1993).

At 6 Months Postgrafting, CD4+ T Cells in RAG-2−/− and RAG-2−/−Class II−/− Recipients Were Indistinguishable with Respect to Cell Size and Expression of CD3, CD4, and NK1.1

To assess the phenotype of CD4+ T cells in recipients, we analyzed cells in LNs and spleen for expression of CD4 versus CD44 (pgp-1) versus NK1.1 and CD4 versus L-selectin (Mel-14). A majority of CD4+ T cells in B6 mice exhibited a virgin phenotype (Bradley et al., 1993): L-selectin^{high} (Figure 6A, top left) and CD44^{low} (Figure 6B, first column). Similarly, at 5 weeks postgrafting, essentially all CD4+ T cells were L-selectin^{high} (Figure 6A, second column) and CD44^{low} (data not shown). Over 6 months postgrafting, a clear shift from an L-selectin^{high} CD44^{low} to an L-selectin^{low}CD44^{high} memory phenotype was observed in RAG-2−/− recipients (Figure 6A, top right and Figure 6B, third column). Similarly, in both groups of recipients, virtually all CD8+ T cells became L-selectin^{low}CD44^{high} during 6 months postgrafting (data not shown). In contrast, most CD4+ T cells in class II−/−RAG-2−/− recipients remained CD44^{low} (Figure 6B, fourth column), but converted to L-selectin^{low} (Figure 6A, bottom right). The presence of CD44^{low} but L-selectin^{low} T cells has been reported previously (Tough and Sprent, 1994). They may be long-lived T cells without having encountered nominal antigens.

Most NK1.1+ and few NK1.1−CD4+ T cells in wild-type mice recognize class I MHC molecules (Bendelac et al., 1994). Accordingly, a substantial fraction of mature

(A) L-selectin (Mel-14) expression in CD4+ T cells in the LNs from B6 (top left), B6class II−/− (bottom left), RAG-2−/−, RAG-2−/−class II−/− recipients at 5 weeks (middle column) and 6 months (right column) postgrafting. Numbers given indicate the percentage of Mel-14^{high} cells over the population range indicated by the horizontal bar.

(B and C) CD4+ T cells from B6, B6class II−/−mice and RAG-2−/− and RAG-2−/−class II−/− recipients at 6 months postgrafting were analyzed by three-color (B; CD4/pgp-1 [CD44]/NK1.1) or by two-color (C; CD4/CD3) flow cytometry. The staining of pgp-1 versus NK1.1 of CD4+ cells from LNs (B, upper row) or spleen (B, lower row) is displayed in the dot plots. The lower row of (C) shows dot plots of CD3 versus CD4; cells present in the lymphoid cell gate (the gate in the dot plots in the upper row) are displayed, and CD3+CD4+ T cells are gated. Conversely, the FSC versus SSC distribution of these CD3+CD4+ T cells is displayed in the dot plots in the upper row of (C). Numbers given in the individual quadrants indicate percentages.

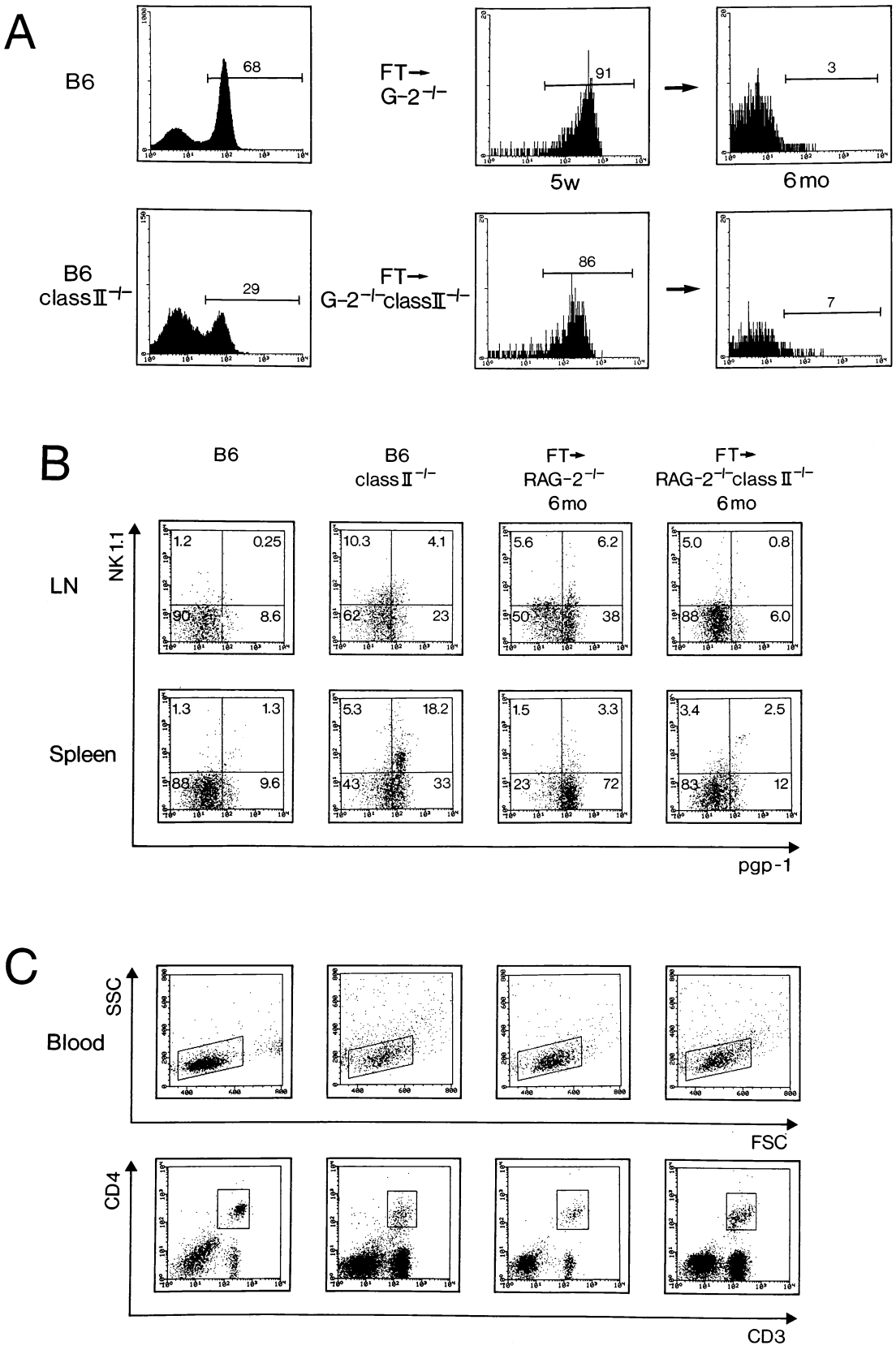

Figure 6. Flow Cytometric Analysis of CD4+ T Cells in B6, B6Class II−/−, RAG-2−/−, and RAG-2−/− Class II−/− Recipients at 6 Months Postgrafting

(*Figure 6 legend continued on previous page*)

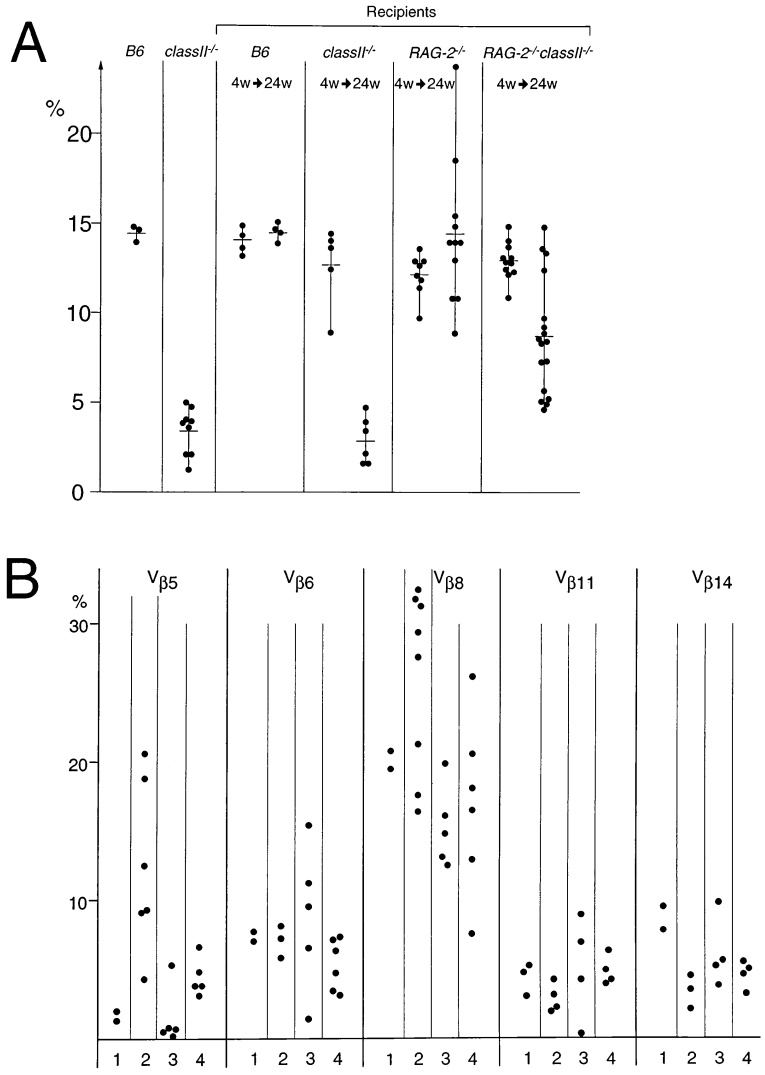

Figure 7. Analysis of Usage of the TCR Vα and Vβ Families by CD4+ T Cells in Lymph Nodes

(A) Percentages of TCR Vα2+ cells among CD4+ T cells are shown. Each dot represents the analysis of an individual mouse, and horizontal bars show the mean value. Each column shows, from left to right, the analysis of the following mice: B6 and B6class II−/− mice and the following four groups of recipients: B6, B6class II−/−, RAG-2−/−, and RAG-2−/−class II−/− recipients. For the recipients, data at 4 and 24 weeks postgrafting are shown.

(B) Each dot shows the usage of TCR Vβ5, Vβ6, Vβ8, Vβ11, and Vβ14 by the CD4+ T cells of an individual mouse. Columns 1–4 show the following groups of recipients: B6 (1), B6class II−/− (2), RAG-2−/− recipients at 6 months postgrafting (3), and RAG-2−/−class II−/− recipients at 6 months postgrafting (4).

CD4+ T cells in B6class II−/− mice (Figure 6B, second column) were NK1.1+ compared with B6 mice (first column). At 6 months postgrafting, few CD4+ T cells were NK1.1+, and CD44highNK1.1+ cells were barely detectable in class II−/−RAG-2−/− recipients (Figure 6B, fourth column). These observations imply that most of the CD4+ T cells that remained in class II−/−RAG-2−/− recipients for 6 months were still restricted by class II MHC molecules.

CD4+ T cells in B6class II−/− mice were normal with respect to the size of cells and expression level of TCR (CD3) and CD4 (Figure 6C, second column). At 6 months postgrafting, CD4+ T cells in RAG-2−/− (Figure 6C, third column) and class II−/−RAG-2−/− recipients (fourth column) did not exhibit any change in the expression level of CD3 or CD4 or in the size of cells. These CD4+ T cells showed a normal proliferative response to stimulation by anti-CD3 antibody (data not shown). In summary, CD4+ T cells in RAG-2−/−class II−/− recipients maintained a normal phenotype for extended periods. Most of these cells remained CD44low and NK1.1− while converting to an L-selectinlow phenotype.

The Usage of the TCR Vα and Vβ Family by CD4+ T Cells in RAG-2−/−Class II−/− Recipients Was Constant for Extended Periods

It has been speculated that peripheral CD4+ T cells in class II−/− mice are reactive to CD1 (Chan et al., 1993) or to class I MHC molecules (Cardell et al., 1995), while a vast majority of CD4+ T cells in wild-type mice are restricted by class II molecules. Since the usage of TCR Vα2 by CD4+ T cells was much lower in B6class II−/− mice than in B6 mice (3.3% versus 14.5% in Figure 7A), TCR Vα2 usage may be useful for assessing the proportion of CD4+ T cells restricted by class II molecules. At 4 weeks postgrafting, the usage of TCR Vα2 by CD4+ T cells was very similar among B6 mice and the four groups of recipients: B6, B6class II−/−, RAG-2−/−, and RAG-2−/−class II−/− recipients (Figure 7A). Interestingly, the usage of TCR Vα2 by CD4+ T cells decreased to 3% in B6class II−/− recipients at 20 weeks postgrafting, but did not significantly decrease in RAG-2−/−class II−/− recipients at 6 months postgrafting. The constant usage of TCR Vα2 and the very low proportion

of NK1.1$^+$CD4$^+$ T cells (Figure 6B) suggest that most CD4$^+$ T cells in RAG-2$^{-/-}$class II$^{-/-}$ recipients were still restricted by class II molecules at 6 months postgrafting. This conclusion is also supported by the observation that CD4$^+$ T cells in B6class II$^{-/-}$ mice do not accumulate with age. By contrast, class I– or CD1-reactive CD4$^+$ T cells seem to be positively selected in B6class II$^{-/-}$ recipients at 20 weeks postgrafting, presumably because donor-type CD4$^+$ T cells had to compete for survival with host-derived CD4$^+$ T cells.

The usage of TCR Vβ families (Figure 7B) was still similar at 6 months postgrafting in RAG-2$^{-/-}$ recipients (Figure 7B, number 3 columns) and RAG-2$^{-/-}$class II$^{-/-}$ recipients (number 4 columns).

Discussion

Recent Thymic Emigrants Can Proliferate without Interactions between the TCR and Class II Molecules

Recent studies have suggested that multiple MHC recognition events are required for positive selection in the thymus (Brandle et al., 1994; Chan et al., 1993; Pircher et al., 1994). This study shows that the presence of class II molecules is not required for the survival of newly generated CD4$^+$ T cells in the periphery. Furthermore, these cells are capable of actively proliferating in the periphery despite the absence of class II molecules. These findings demonstrate that the engagement of the TCR with class II molecules, which is essential for positive selection of CD4$^+$ T cells in the thymus, is not required for the survival of peripheral CD4$^+$ T cells.

It appears that the proliferation of recent thymic emigrants is controlled by the total number of peripheral CD4$^+$ T cells. Previous in vivo BrdU or fluorescein isothiocyanate (FITC) labeling experiments revealed that most recent thymic emigrants in euthymic mice did not divide (Scollay et al., 1980; Tough and Sprent, 1994). In contrast, virtually all recent thymic emigrants were actively proliferating in the periphery in RAG-2$^{-/-}$ and class II$^{-/-}$RAG-2$^{-/-}$ recipients. Likewise, both donor and host-derived newly generated CD4$^+$ T cells in class II$^{-/-}$B6 recipients seemed to proliferate actively in the periphery and fill the empty CD4$^+$ T cells compartment quickly. The active proliferation of newly generated CD4$^+$ T cells in CD4$^+$ T cell–deficient mice, but not in wild-type mice, shows that it is not TCR occupancy that stimulates the proliferation of newly generated CD4$^+$ T cells.

CD4$^+$ T Cells Can Proliferate in the Absence of Antigenic Stimulation

We speculated that the proliferation of CD4$^+$ T cells occurred primarily in the absence of antigenic stimulation not only at 5 weeks, but also at 10 and 24 weeks postgrafting. However, we could not completely exclude the involvement of class II molecules in the activation of CD4$^+$ T cells in class II$^-$ recipients. In fact, peripheral T cells in class II$^-$ recipients may be able to migrate to the class II$^+$ thymic graft. However, this migration is normally restricted to activated T cells (Agus et al., 1991). In addition, the turnover of migrating T cells seems to be very slow (Agus et al., 1991) and would not likely account for the number of BrdU-labeled CD4$^+$ T cells in class II$^{-/-}$RAG-2$^{-/-}$ recipients. Class II$^+$ cells were not detectable in the periphery of class II$^-$ recipients at 10 and 24 weeks postgrafting (data not shown).

We speculated that, although RAG-2$^{-/-}$ and class II$^{-/-}$RAG-2$^{-/-}$ recipients are immunodeficient, the rapid turnover of T cells observed in these recipients was not caused by infection or pathological inflammation for the following reasons: first, active cycling of CD4$^+$ T cells was observed to the same extent in the recipients, regardless of whether or not the recipients contained class II$^+$ antigen-presenting cells; second, T cells in healthy wild-type mice (Balb/c) have been shown to divide actively, and 17% of splenic CD4$^+$ T cells incorporated BrdU over 5 days (Sprent et al., 1991); third, percentages of BrdU-labeled T cells were consistent among individual recipients and remained constant at 10 and 24 weeks postgrafting; fourth, the recipients exhibited no signs of infection (no LN swelling or splenomegaly); and, fifth, the constant usage of TCR Vβ families argues against the involvement of superantigens (Janeway, 1991; Herman et al., 1991) or the expansion of oligo-clonal T cells.

It has been speculated that most CD4$^+$ T cells were still restricted by class II molecules in class II$^{-/-}$RAG-2$^{-/-}$ recipients, even at 6 months postgrafting, for the following reasons. First, peripheral CD4$^+$ T cells in B6class II$^{-/-}$ mice, which are reactive to the CD1 or class I MHC molecules, do not increase in number with age, although their number is much lower than in normal animals. Hence, it is not likely that CD4$^+$ T cells reactive to the CD1 or class I MHC molecules had increased in number in class II$^{-/-}$RAG-2$^{-/-}$ recipients. Second, the relatively high usage of TCR Vα2 and the low proportion of NK1.1$^+$CD4$^+$ T cells suggest that CD1- or MHC class I–reactive CD4$^+$ T cells were not a major subset in class II$^{-/-}$RAG-2$^{-/-}$ recipients. Hence, antigenic stimulation by MHC class I or CD1 molecules does not account for the proliferation of CD4$^+$ T cells in class II$^{-/-}$RAG-2$^{-/-}$ recipients at 10 and 24, as well as 5, weeks postgrafting.

The total number of CD4$^+$ T cells appears to regulate the proliferation of CD4$^+$ T cells, not only at 5 weeks but also at 10 and 24 weeks postgrafting. Since the rate of turnover of CD4$^+$ T cells was very similar in RAG-2$^{-/-}$ and class II$^{-/-}$RAG-2$^{-/-}$ recipients at 10 and 24 weeks postgrafting, we speculated that the turnover of CD4$^+$ T cells in class II$^+$ as well as class II$^-$ recipients was mostly independent of antigenic stimulation. In addition, the number of proliferating cells and the death rate of CD4$^+$ T cells should be identical in RAG-2$^{-/-}$ recipients, suggesting the presence of a homeostatic mechanism that maintains a constant number of CD4$^+$ T cells in each animal. This mechanism, however, did not seem to work perfectly in class II$^{-/-}$RAG-2$^{-/-}$ recipients, causing a very slow decline in the number of CD4$^+$ T cells.

In summary, we believe that antigenic stimulation plays little role in the regulation of the cycling of CD4$^+$ T cells in the recipients. However, our data do not exclude the involvement of weak interactions between the TCR and MHC molecules. The total number of CD4$^+$ T cells plays an important role in regulating the cycling of

$CD4^+$ T cells; unknown humoral factors, whose concentration is correlated with the total number of $CD4^+$ T cells, may be responsible for this type of cell division.

Weak Interactions between the TCR and Class II Molecules Are Necessary to Maintain the Size of the $CD4^+$ T Cell Compartment and the TCR Repertoire of $CD4^+$ T Cells

Since the total number of $CD4^+$ T cells gradually declined in class $II^{-/-}$RAG-$2^{-/-}$ recipients, proliferation of $CD4^+$ T cells must be accompanied by the death of other $CD4^+$ T cells. The gradual decline in the number of $CD4^+$ T cells despite active proliferation is reminiscent of the rapid turnover of $CD4^+$ T cells observed in HIV patients (Ho et al., 1995; Wei et al., 1995). The slow decrease in the number of $CD4^+$ T cells in class $II^{-/-}$RAG-$2^{-/-}$ recipients indicates that the presence of class II molecules affects the life span of $CD4^+$ T cells.

T cells are capable of surviving for prolonged periods without receiving antigenic stimulation. In fact, upon adoptive transfers of naive $CD8^+$ T cells carrying a transgenic TCR specific for the male peptide (HY) into female nude mice, numbers of injected T cells remained constant over 101 days postgrafting (Bruno et al., 1995; von Boehmer and Hafen, 1993). This experiment, however, did not address the role of weak interactions between MHC class I molecules and the TCR in the regulation of the turnover of T cells. The present data show that engrafted $CD4^+$ T cells in class $II^{-/-}$RAG-$2^{-/-}$ recipients gradually decreased in number over 6 months. These findings suggest that weak interactions between the TCR and class II molecules affect the maintenance of the number of peripheral $CD4^+$ T cells and the size of the TCR repertoire. Presumably, self-peptides presented by class II molecules provide continuous low level TCR stimulation, and rescue $CD4^+$ T cells from cell death. The vast size of the TCR repertoire expressed by the pool of peripheral $CD4^+$ T cells may reflect the significantly heterogeneous self-peptides presented by class II molecules.

Experimental Procedures

Mice

B6(Ly5.1) and B6class $II^{-/-}$ (Köntgen et al., 1993) mice were obtained from IFFA–Credo (France) and Füllinsdorf (Switzerland), respectively. Breeding stocks B6Ly5.2 and RAG-$2^{-/-}$ mice (Shinkai et al., 1992) were provided by Drs. C. Reeder (National Cancer Institute, Frederick, MD) and F. Alt (The Children's Hospital, Boston, MA), respectively. We backcrossed RAG-$2^{-/-}$ mice twice with B6class $II^{-/-}$ and obtained class $II^{-/-}$RAG-$2^{+/-}$ mice. We then intercrossed these mice to obtain class $II^{-/-}$RAG-$2^{-/-}$ mice. Genotypes of offspring were determined by examining the tail DNA as described before (Köntgen et al., 1993; Shinkai et al., 1992).

Antibodies for Flow Cytometry

The following primary antibodies were used in this study: FITC- or biotin-conjugated 104-2.1 (anti-Ly5.1; Shen, 1981), FITC- or biotin-conjugated A20-1.7 (anti-Ly5.2; Shen, 1981), FITC- or biotin-conjugated GK 1.5 (anti-CD4; Dialynas et al., 1983; Becton Dickinson), FITC- or biotin-conjugated 53-6.7 (anti-CD8; Ledbetter and Herzenberg, 1979; Pharmingen), biotin-conjugated 145-2C11 (anti-CD3; Leo et al., 1987; Pharmingen), FITC-conjugated IM7 (anti-CD44 [pgp-1]; Budd et al., 1987; Pharmingen), biotin-conjugated PK136 (anti-NK1.1; Koo and Peppard, 1984; Pharmingen), biotin-conjugated MEL-14 (anti-L-selectin [Ly-22]; Fink et al., 1985; Pharmingen), FITC-conjugated B20.1 (anti-TCR Vα2; Pircher et al., 1992; Pharmingen), FITC-conjugated B20.1 (anti-TCR Vβ5.1, 5.2; Pircher et al., 1992; Pharmingen), FITC-conjugated RR4-7 (anti-TCR Vβ6; Pircher et al., 1992; Pharmingen), FITC-conjugated MR5-2 (anti-TCR Vβ8.1, 8.2; Kanagawa, 1988; Pharmingen), FITC-conjugated RR3-15 (anti-TCR Vβ11; Bill et al., 1989; Geo et al., 1989; Pharmingen), and FITC-conjugated MR14-2 (anti-TCR Vβ14; Liao et al., 1989; Pharmingen). Second-step reagent was streptavidin–tricolor (Caltag).

Transplantation of Fetal Thymus

To obtain day 15 fetal mouse, we intercrossed B6Ly5.2 mice and counted the time of the vaginal plug (10:00 A.M.) as day 0 of pregnancy. Both thymic lobes were removed from fetal mice. For thymus grafting, 10- to 14-week-old mice were given antibiotics (neomycin sulfate; Sigma) 1 day before and after the surgery through drinking water (1.2 g per liter). They were anesthetized with Avertin (2.5% solution, 10 μl per gram body weight) as described previously (Hogan et al., 1986). A small incision (<5 mm) was made in the peritoneal cavity, the left kidney was exposed under a stereo microscope, and two thymic lobes from an individual fetus were positioned under the kidney capsule with the use of microdissection forceps. Subsequently, the wound was closed using surgical sutures.

Immunofluorescence Staining and Flow Cytometric Analysis

For phenotypic analysis, single cell suspensions from a pool of cervical, auxiliary, inguinal, and mesenteric LNs and spleen (red blood cells lysed) were prepared in PBS plus 2% FCS. Peripheral blood was taken from tail and immediately mixed with 10 μl of heparin (Roche, Switzerland). To isolate nucleated cells, we overlaid the whole blood on Ficoll–Paque (Pharmacia) and spun it at 1500 rpm for 20 min at room temperature. Nucleated cells at the intermediate phase were washed twice with PBS and suspended in PBS plus 2% FCS. Cells (1×10^6 to 3×10^6) were incubated with purified MAbs at 5–10 μg/ml in PBS with 5% FCS for 15–30 min on ice and washed once in PBS, 5% FCS. Flow cytofluorometric analysis was performed on a FACscan (Becton Dickinson). Dead cells were excluded using forward- and side-scatter parameters to examine the percentage of nucleated cells. Cells presented in the lymphocyte gate, as defined by light scatter (Kisielow et al., 1988), were analyzed to measure percentages of $CD4^+$ and $CD8^+$ T cells among nucleated cells. Fluorescence data are displayed as logarithmic histograms or dot plots using LYSYS software (Becton Dickinson).

BrdU Labeling In Vivo, Sorting of $CD4^+$ and $CD8^+$ T Cells, and Detection of $BrdU^+$ Cells by Flow Cytometry

Mice were given BrdU (Sigma) through drinking water at 1 mg/ml for 5 days. BrdU-containing water was replaced with freshly prepared water twice during the 5 day period. For pulse–chase experiments, the mice were subsequently given normal drinking water for 2 weeks. The mice were killed, and single cell suspension of LNs and spleen (red blood cells lysed) were prepared in PBS with 2% FCS. Sorter-purified cells ($CD4^+CD8^-$ and $CD4^-CD8^+$) were treated as described before (Huesmann et al., 1991). In brief, cell sorter–purified T cells were permeabilized by addition of 500 μl of 70% ethanol in water at room temperature to cell pellets. Cells were incubated overnight at 4°C and washed twice with PBS before addition of 500 μl of 3 N HCl, 0.5% Tween 20. After 20 min, the cells were pelleted, supernatants were removed, and 500 μl of 0.1 M sodium tetraborate was added. Cells were washed twice in PBS, 0.5% Tween 20 and incubated with 40 μl of PBS plus 20 μl of FITC-labeled anti-BrdU antibody (Becton Dickinson). Incubation, washes, and cytofluorometric analysis were performed as described above. Unlabeled cells stained with anti-BrdU MAb and labeled cells without staining by anti-BrdU MAb were used as negative controls.

Thymectomy of Mice

The thymus was removed from 6-week-old B6 and B6class $II^{-/-}$ mice as previously described (Tough and Sprent, 1994).

Acknowledgments

Correspondence should be addressed to S. T. We thank Gholam Reza Dastoornikoo for excellent technical help. We would like to

thank Drs. F. Alt (The Children's Hospital, Boston, MA) and C. Reeder (National Cancer Institute, Frederick, MD) for generously providing RAG-2$^{-/-}$ and B6Ly5.2 congenic mice, respectively; E. Wagner and the Animal Facility staff (Basel) are acknowledged for their care in maintenance of mouse colonies; K. Hafen for advice on thymectomy; M. Dessing and S. Meyer for cell sorter expertise; and H. Stahlberger for artwork. We thank Drs. Diane Mathis, Christophe Benoist, Jörg Kirberg, B. A. Constantin, and Jean-Marie Buerstedde for discussions and critical reading of the manuscript. The *Bayer*-chair department is supported by Bayer Japan. The Basel Institute for Immunology was founded and is supported by F. Hoffmann La Roche, Basel.

Received November 23, 1995; revised July 18, 1996.

References

Agus, D.B., Surh, C.D., and Sprent, J. (1991). Reentry of T cells to the adult thymus is restricted to activated T cells. J. Exp. Med. *173*, 1039–1046.

Ashton-Rickardt, P.G., Bandeira, A., Delaney, J.R., Van Kaer, L., Pircher, H.-P., Zinkernagel, R.M., and Tonegawa, S. (1994). Evidence for a different avidity model of T cell selection in the thymus. Cell *76*, 651–663.

Bendelac, A., Killeen, N., Littman, D.R., and Schwartz, R.H. (1994). A subset of CD4$^+$ thymocytes selected by MHC class I molecules. Science *263*, 1774–1778.

Bill, J., Kanagawa, O., Woodland, D., and Palmer, E. (1989). The MHC molecule I-E is necessary but not sufficient for the clonal deletion of Vβ11 bearing T cells. J. Exp. Med. *169*, 1405–1419.

Bradley, L.M., Croft, M., and Swain, S.L. (1993). T-cell memory: new perspectives. Immunol. Today *14*, 197–199.

Brandle, D., Muller, S., Muller, C., Hengartner, H., and Pircher, H. (1994). Regulation of RAG-1 and CD69 expression in the thymus during positive and negative selection. Eur. J. Immunol. *24*, 145–151.

Bruno, L., Kirberg, J., and von Boehmer, H. (1995). On the cellular basis of immunological T cell memory. Immunity *2*, 37–43.

Budd, R., Cerottini, J., Horvath, C., Bron, C., Pedrazzini, T., Howe, R., and MacDonald, R. (1987). Distinction of virgin and memory T lymphocytes. J. Immunol. *138*, 3120–3129.

Callahan, J.E., Kappler, J.W., and Marrack, P. (1993). Unexpected expansion of CD8-bearing cells in old mice. J. Immunol. *151*, 6657–6669.

Cardell, S., Tangri, S., Chan, S., Kronenberg, M., Benoist, C., and Mathis, D. (1995). CD1-restricted CD4$^+$ T cells in major histocompatibility complex class II-deficient mice. J. Exp. Med. *182*, 1–12.

Chan, S.H., Cosgrove, D., Waltzinger, C., Benoist, C., and Mathis, D. (1993). Another view of the selective model of thymocyte selection. Cell *73*, 225–236.

Cosgrove, D., Gray, D., Dierich, A., Kaufman, J., Lemeur, M., Benoist, C., and Mathis, D. (1991). Mice lacking MHC class II molecules. Cell *66*, 1051–1066.

Dialynas, D.P., Wilde, D.B., Marrack, P., Pierres, A., Wall, K.A., Havran, W., Otten, G., Loken, M.R., Pierres, M., Kappler, J., and Fitch, F.W. (1983). Characterization of the murine antigenic determinant, designated L3T4$_a$, recognized by monoclonal antibody GK1.5: expression of L3T4$_a$ by functional T cell clones appears to correlate primarily with class II MHC antigen reactivity. Immunol. Rev. *74*, 29–56.

Fink, P., Gallatin, W., Reichert, R., Butcher, E., and Weissman, I. (1985). Homing receptor-bearing thymocytes, an immunocompetent cortical subpopulation. Nature *313*, 233–235.

Fowlkes, B., and Schweighoffer, E. (1995). Positive selection of T cells. Curr. Opin. Immunol. *7*, 188–195.

Frey, J.R., Ernst, B., Surh, C.D., and Sprent, J. (1992). Thymus-grafted SCID mice show transient thymopoiesis and limited depletion of Vβ11$^+$ T cells. J. Exp. Med. *175*, 1067–1071.

Geo, E., Kanagawa, O., and Sprent, J. (1989). Capacity of unprimed CD4$^+$ and CD8$^+$ T cells expressing Vβ11 receptors to respond to I-E alloantigens *in vivo*. J. Exp. Med. *170*, 1947–1957.

Grusby, M.J., Johnson, R.S., Papaioannou, V.E., and Glimcher, L.H. (1991). Depletion of CD4$^+$ T cells in major histocompatibility complex classII-deficient mice. Science *253*, 1417–1420.

Herman, A., Kappler, J.W., Marrack, P., and Pullen, A. (1991). Superantigens: mechanism of T cell stimulation and role in immune responses. Annu. Rev. Immunol. *9*, 745–772.

Ho, D.D., Neumann, A.U., Perelson, A.S., Chen, W., Leonard, J.M., and Markowitz, M. (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature *373*, 123–126.

Hogan, B., Constantini, F., and Lacy, E. (1986). Manipulating the Mouse Embryo: A Laboratory Manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), p. 271.

Hogquist, K.A., Gavin, M.A., and Bevan, M.J. (1993). Positive selection of CD8$^+$ T cells induced by major histocompatibility complex binding peptides in fetal thymic organ culture. J. Exp. Med. *177*, 1469–1473.

Huesmann, M., Scott, B., Kisielow, P., and von Boehmer, H. (1991). Kinetics and efficacy of positive selection in the thymus of normal and T cell receptor transgenic mice. Cell *66*, 533–540.

Jameson, S.C., Hogquist, K.A., and Bevan, M.J. (1995). Positive selection of thymocytes. Annu. Rev. Immunol. *13*, 93–126.

Janeway, C.A., Jr. (1991). Selective elements for the Vβ region of the T cell receptor: MIs and the bacterial toxic mitogens. Adv. Immunol. *50*, 1–53.

Kanagawa, O. (1988). Antibody-mediated activation of T cell clones as a method for screening hybridomas producing antibodies to the T cell receptor. J. Immunol. Meth. *110*, 169–178.

Kirberg, J., Bruno, L., and von Boehmer, H. (1993). CD4$^+$CD8$^-$ help prevents rapid deletion of CD8$^+$ T cells after a transient response to antigen. Eur. J. Immunol. *23*, 1963–1967.

Kisielow, P., and von Boehmer, H. (1995). Development and selection of T cells: facts and puzzles. Adv. Immunol. *58*, 87–209.

Kisielow, P., Blüthmann, H., Staerz, U.D., Steinmetz, M., and von Boehmer, H. (1988). Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4$^+$8$^+$ thymocytes. Nature *333*, 742–746.

Köntgen, F., Süss, G., Stewart, C., Steinmetz, M., and Bluethmann, H. (1993). Targeted disruption of the class II Aa gene in C57BL/6 mice. Int. Immunol. *5*, 957–964.

Koo, G., and Peppard, J. (1984). Establishment of monoclonal anti-NK1.1 antibody. Hybridoma *3*, 301–303.

Ledbetter, J.A., and Herzenberg, L.A. (1979). Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. *47*, 63–90.

Leo, O., Foo, M., Sachs, D.H., Samelson, L.E., and Bluestone, J.A. (1987). Identification of a monoclonal antibody specific for a murine T3 polypeptide. Proc. Natl. Acad. Sci. USA *84*, 1374–1378.

Liao, N., Maltzman, J., and Raulet, D.J. (1989). Positive selection determines T cell receptor V$_{\beta 14}$ gene usage by CD8$^+$ T cells. J. Exp. Med. *170*, 135–143.

Lo, D., and Sprent, J. (1986). Identity of cells that imprint H-2-restricted T-cell specificity in the thymus. Nature *319*, 672–675.

Mombaerts, P., Iacomini, J., Johnson, R.S., Herrup, K., Tonegawa, S., and Papaioannou, V.E. (1992). RAG-1-deficient mice have no mature B and T lymphocytes. Cell *68*, 869–877.

Pircher, H., Rebal, N., Groettrup, M., Gregoire, C., Speiser, D.E., Happ, M.P., Palmer, E., Zinkernagel, M., Hengartner, H., and Malissen, B. (1992). Preferential positive selection of Vα2$^+$/CD8$^+$ T cells in mouse strains expressing both H-2^k and T cell receptor Vαa haplotype: determination with Vα2-specific monoclonal antibody. Eur. J. Immunol. *22*, 399–404.

Pircher, H., Ohashi, P.S., Boyd, R.L., Hengartner, H., and Brduscha, K. (1994). Evidence for a selective and multi-step model of T cell differentiation: CD4$^+$CD8^{low} thymocytes selected by a transgenic T cell receptor on major histocompatibility complex class I molecules. Eur. J. Immunol. *24*, 1982–1987.

Schumacher, T.N.M., and Ploegh, H.L. (1994). Are MHC-bound peptides a nuisance for positive selection? Immunity *1*, 721–723.

Scollay, R., Butcher, E., and Weissman, I.L. (1980). Thymus cell migration: quantitative studies of cellular traffic from the thymus to the periphery in mice. Eur. J. Immunol. *10*, 210–218.

Shen, F.-W. (1981). Monoclonal antibodies to mouse lymphocyte differentiation alloantigens. In Monoclonal Antibodies and T Cell Hybridomas, G. Haemmerling, U. Haemmerling, and J.F. Kearney, eds. (Amsterdam: Elsevier North Holland/Biomedical Press), pp. 25–31.

Shinkai, Y., Rathbun, G., Lam, K.-P., Oltz, E.M., Stewart, V., Mendelsohn, M., Charron, J., Datta, M., Young, F., Stall, A.M., and Alt, F.W. (1992). RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. Cell *68*, 855–867.

Shortman, K., Egerton, M., Spangrude, G.J., and Scollay, R. (1990). The generation and fate of thymocytes. Semin. Immunol. *2*, 3–12.

Sprent, J. (1993). Lifespans of naive, memory and effector lymphocytes. Curr. Opin. Immunol. *5*, 433–438.

Sprent, J., Schaefer, M., Hurd, M., Surh, C., and Ron, Y. (1991). Mature murine B and T cells transferred to SCID mice can survive indefinitely and many maintain a virgin phenotype. J. Exp. Med. *174*, 717–728.

Tough, D.F., and Sprent, J. (1994). Turnover of naive- and memory-phenotype T cells. J. Exp. Med. *179*, 1127–1135.

von Boehmer, H., and Hafen, K. (1993). The lifespan of naive α/β T cells in secondary lymphoid organs. J. Exp. Med. *177*, 891–896.

Wei, X., Ghosh, S.K., Taylor, M.E., Johnson, V.A., Emini, E.A., Deutsch, P., Lifson, J.D., Bonhoeffer, S., Nowak, M.A., Hahn, B.H., Saag, M.s., and Shaw, G.M. (1995). Viral dynamics in human immunodeficiency virus type 1 infection. Nature *373*, 117–122.