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Memory Phenotype of CD8+ T Cells in MHC Class Ia-Deficient Mice

Zoran Kurepa,2* Jie Su,* and James Forman3*

B6.Kb−/−Dd−/− mice are devoid of class Ia but express normal levels of class Ib molecules. They have low levels of CD8 T cells in both the thymus as well as peripheral T cell compartments. Although the percentage of splenic CD8α T cells is increased in these animals, ~90% of CD8 T cells are CD8αβ. In contrast to B6 animals, most of the CD8 T cells from these mice have a memory phenotype (CD44highCD122highCD62Llow) including both CD8αβ and CD8αα subsets. In the thymus of B6.Kb−/−Dd−/− animals, there is a decrease in the percentage of SP CD8 T cells, although most are CD44low, similar to that seen in B6 mice. The spleens from day 1-old B6 and B6.Kb−/−Dd−/− mice have a relatively high proportion of CD44highCD62Llow CD8 T cells. However, by day 28 most CD8 T cells in B6 mice have a naive phenotype while in B6.Kb−/−Dd−/− mice the memory phenotype remains. Unlike CD44high cells that are found in B6 animals, most CD44high cells from B6.Kb−/−Dd−/− mice do not secrete IFN-γ rapidly upon activation. The paucity of CD8 T cells in B6.Kb−/−Dd−/− mice might be due in part to their inability to undergo homeostatic expansion. Consistent with this, we found that CD8 T cells from these animals expand poorly in X-irradiated syngeneic hosts compared with B6 CD8 T cells that respond to class Ia Ags. We examined homeostatic expansion of B6 CD8 T cells in single as well as double class Ia knockout mice and were able to estimate the fraction of cells reactive against class Ia vs class Ib molecules. The Journal of Immunology, 2003, 170: 5414–5420.

The murine MHC encodes both classical (class Ia) and non-classical (class Ib) molecules. Although the number of class I genes in the Q, T, and M regions of the murine MHC is large, only a few are known to encode cell membrane molecules that associate with β2-microglobulin (β2-M) and thus likely to present Ag to the TCR on CD8 T cells (1). Unlike class Ia molecules, the expression of class Ib molecules is relatively low and sometimes limited in tissue distribution. In addition, it has been noted that some class Ib molecules normally bind a limited array of peptides (Qa-1) (2), formylated peptides (M3) (3), or no peptides (T10, T22) (4), while others such as Q7 and Q9, which encode Qa-2 molecules, present a wide array of peptides (5).

B6.Kb−/−Dd−/− mice represent a powerful tool for investigating the potential of CD8 cells specific for non-class Ia Ags. These mice lack expression of class Ia K and Dβ1 H chains but show normal expression of class Ib molecules (6). Although the number of CD8 T cells is greatly reduced in these mice, the CD8 cells function effectively to mediate immune responses. Furthermore, the diversity of the CD8+ T cell repertoire selected only on MHC class Ib molecules is very similar, with respect to the usage of various Vα and Vβ rearrangements, to the one selected on both MHC class Ia and class Ib molecules (7). Thus, CD8 cells from these animals respond to pathogens such as *Listeria monocytogenes* (LM) and generate Ag-specific CTL restricted by the class Ib molecules M3 and Qa-1b (8). Although the kinetics of their response to LM is altered following primary infection, they do generate memory cells that provide protection against LM infection (9, 10). Furthermore, CD8 cells from these mice mount strong alloresponses against class Ia molecules including Kb and Dd (8, 11).

In a previous study, we noted that most CD8 T cells from B6.Kb−/−Dd−/− spleens are CD44high (8). This phenotype is conventionally used to identify CD8 T memory cells (12) and thus could reflect the possibility that most class Ib-reactive cells in adult mice have previously encountered Ags, either representing nonpathogens or self-Ags. The CD44high phenotype has also been noted on cells undergoing homeostatic expansion (13). It is a marker that once acquired is thought to remain stable on such cells (14). Since the number of peripheral CD8 cells is decreased in B6.Kb−/−Dd−/− animals and thus presents a CD8 lymphopenic environment, this may in part account for this phenotype.

In this report, we have characterized the CD8 T cells resident in B6.Kb−/−Dd−/− animals along with the ontogeny of this CD44 phenotype. We further investigate the proportion of CD8 T cells from normal mice that respond through homeostatic expansion in single class Ia-deficient as well as mice lacking both Kb−/− and Dd−/− molecules.

Materials and Methods

**Mice**

B6.Kb−/−Dd−/−, B6.Kb−/−, B6.Dd−/−, C57BL/6, B6.β2-M−/−, and B6.Thy1.1 mice were bred and maintained in animal colonies at the University of Texas Southwestern Medical Center (Dallas, TX) under specific pathogen-free conditions. B6.Kb−/−Dd−/−, B6.Kb−/−, and B6.Dd−/− mice were generated as previously described (6) and were a generous gift from F. Lemmonier (Institute Pasteur, Paris, France). B6.Kb−/−Dd−/− mice were a kind gift from Dr. C. Suth (The Scripps Research Clinic, San Diego, CA).

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4 Abbreviations used in this paper: β2-M, β2-microglobulin; LM, *Listeria monocytogenes*; SP, single positive.
Adoptive transfer of CFSE-labeled cells

For cell transfers, single-cell suspensions were prepared from freshly isolated spleens. After lysing RBCs using erythrocyte lysing buffer (R&D Systems, Minneapolis, MN), splenocytes were enriched for T cells by passing them through a nylon wool column. The cells were labeled with CFSE by incubation with 1 μM CFSE (Molecular Probes, Eugene, OR) for 15 min at 37°C in balanced salt solution followed by quenching the unlabeled CFSE by adding excess amounts of FCS and washing. Recipient mice were injected i.v. with 1×10⁶ CFSE-labeled cells. Where necessary, recipient mice were subjected to gamma-irradiation with 650 rad 24 h before cell transfer.

Flow cytometric analysis and Abs

Spleen, thymic, and lymph node cells (1×10⁶) were stained for 30 min at 4°C with the appropriate concentrations of mAbs in PBS containing 1% FCS and 0.1% NaN₃. For flow cytometric analysis, anti-CD3ε FITC (145-2C11), anti-CD4 PE (RM4-5), anti-CD8α (53-6.7), anti-CD8β PE (53-5.8), anti-CD44 PE, allophycocyanin, or biotin (IM7), anti-CD122 biotin (TM-β1), anti-CD90.1 biotin (HIS51), anti-CD90.2 biotin (30-H12), anti-CD62L biotin (MEL-14), anti-CD16/CD32 (2.4G2), anti-IFN-γ allophycocyanin, and anti-CD24 (M1/69) PE were purchased from BD PharMingen. Following two washes with PBS/FCS/NaNa₃, cells were incubated with second-layer reagents: streptavidin-PerCP, streptavidin-allophycocyanin, or streptavidin-CyChrome (BD PharMingen). After two more washes, cells were acquired using FACScan or FACSColor flow cytometers (BD Biosciences, Mountain View, CA). Data files were analyzed using CellQuest software (BD Biosciences, Mountain View, CA).

Cell proliferation analysis

Donor CFSE-labeled cells were identified in normal and irradiated hosts and analyzed for CFSE expression to establish rounds of cell division in the host. Calculation of percent cells in each round was performed according to an established protocol (15).

Intracellular IFN-γ staining

Freshly isolated spleen cells were cultured with or without 500 ng/ml ionomycin and 25 ng/ml PMA. At varying times, the cells were treated with Golgiplug containing brefeldin A (BD PharMingen), harvested, and stained for cell surface markers. The cells were then fixed, permeabilized, and stained for intracellular IFN-γ.

Results

Phenotype of CD8⁺ T cells from MHC class Ia-deficient mice

We previously noted that the majority of CD8⁺ T cells from naive B6.Kb⁻/⁻/Db⁻/⁻ mice display a phenotype typical of memory cells with >60% of CD8⁺ T cells being CD44high (8). To extend these findings, we examined these cells for additional markers characteristic of memory cells. It has been reported that once formed, CD8⁺ T cells can survive indefinitely but their survival requires contact with cytokines, in particular IL-15 (16). Consequently, we further analyzed these cells for the expression of the IL-15 receptor β-chain (CD122). As shown in Fig. 1b, 52% of CD8⁺ T cells from adult B6.Kb⁻/⁻/Db⁻/⁻ mice are CD122⁺. Virtually all of these cells are CD44high, although there are also 24% CD44highCD122⁻ cells. In contrast, only 17% of CD8⁺ T cells in B6 mice are CD122⁺CD44high (Fig. 1a). We also examined these cells for the expression of the lymph node-homing receptor CD62L which is decreased on activated memory cells (12). In MHC class Ia-deficient mice, 80% of CD8 cells are CD62Llow, whereas in B6 splenocytes only 40% of CD8⁺ T cells are CD62Llow (Fig. 1, c and d).

To determine whether this phenotype is characteristic of other class Ia-deficient strains, we examined CD8 cells from both (β₂M⁻/⁻) and Tap-1⁻/⁻ mice. Both of these strains have ~1% CD8 cells in their splenic compartment (17, 18). More than 90% of the CD8 cells from these strains are CD44high, 45–48% are CD122high and 40–64% are CD62Llow (Table I).

It has been recently reported that most of the CD8⁺ cells in β₂M⁻/⁻ animals do not express CD8β, but express high levels of CD8α (19). In addition, CD8αε T cells have been shown to be CD44high (20). Therefore, we determined the percentage of CD8⁺ T cells in B6.Kb⁻/⁻/Db⁻/⁻ mice that are CD8αε by staining with both CD8α and CD8β Abs as well as for CD3 expression (Fig. 2a). In the CD8 T cell compartment, adult B6 mice have 2–3% CD8αε cells in their spleen. This percentage is elevated in the B6.Kb⁻/⁻/Db⁻/⁻ mice to ~10% of CD3⁺ CD8⁺ cells. We also compared the level of CD44 expression of the CD8αβ and CD8αε cells. Although all CD8αε cells are CD44high in both strains, CD8αβ cells are mostly CD44low from B6 mice but CD44high from B6.Kb⁻/⁻/Db⁻/⁻ mice (Fig. 2, b and c). More than 98 and 92% of CD8 αβ cells from B6 and B6.Kb⁻/⁻/Db⁻/⁻ mice are TCRV₆, respectively (data not shown). On an absolute cell number basis, CD8αε cells are not increased in the B6.Kb⁻/⁻/Db⁻/⁻ animals. Thus, B6 and B6.Kb⁻/⁻/Db⁻/⁻ mice have 1.24 ± 1.1 × 10⁶ and 0.85 ± 0.2 × 10⁶ (n = 3) CD8αε cells, respectively, in their spleen,
which is consistent with the findings that these cells can be selected on class I b Ags (21).

## Expression of memory markers on CD8 T cells from Tap-1−/− and β2-M−/− strains

<table>
<thead>
<tr>
<th>Marker</th>
<th>Tap-1−/−</th>
<th>β2-M−/−</th>
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<tbody>
<tr>
<td>CD44&lt;sup&gt;high&lt;/sup&gt;</td>
<td>90.4 ± 1.1</td>
<td>96.0 ± 3.5</td>
</tr>
<tr>
<td>CD122&lt;sup&gt;high&lt;/sup&gt;</td>
<td>48.3 ± 8.4</td>
<td>44.8 ± 12.3</td>
</tr>
<tr>
<td>CD62L&lt;sup&gt;low&lt;/sup&gt;</td>
<td>64.2 ± 7.5</td>
<td>39.6 ± 4.5</td>
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* Spleen cells were stained for the above markers. n = 3.

## Ontogeny of CD44 and CD62L expression on CD8 T cells from B6 and B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> mice

All CD8<sup>+</sup> T cells in a MHC class Ia-deficient host are presumably selected on molecules other than class Ia. As a result, the CD8αβ T cell number is reduced by 80–90% in B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> mice when compared with C57BL/6 mice (6). Therefore, naive T cells in these mice are circulating in a CD8 lymphopenic environment which could result in their expansion and acquisition of memory cell markers such as CD44<sup>high</sup> (13). The lymphopenic environment could in part be due to the paucity of single-positive (SP) CD8 cells present in the thymus as B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> mice have <~1% of this cell population compared with 3–6% in B6 animals (Fig. 3, a and b). Furthermore, most of the CD8<sup>+</sup> CD4<sup>+</sup> cells in the B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> thymus are CD44<sup>low</sup>, similar to that seen in B6 mice. This is noted when the SP cells are gated on either CD24<sup>high</sup> or TCRV<sup>β</sup><sup>high</sup>, both markers for the mature phenotype (Fig. 3, c–f) (22).

Since most splenic CD8<sup>+</sup> T cells in B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> are CD44<sup>high</sup>, we determined when these cells acquire this phenotype. Spleen cells from day 1-old C57BL/6 mice displayed a relatively high ratio of CD44<sup>hlow</sup>CD44<sup>low</sup> CD8 cells that rapidly decreased with age (Fig. 4a). By day 28, the ratio was close to that seen in adult mice. In contrast, day 1 spleen cells from B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> mice showed a high ratio of CD44<sup>hlow</sup>CD44<sup>low</sup> CD8 cells which remained high not only at day 56 (Fig. 4b) but continued that phenotype throughout adult life (data not shown). A second marker for T cell memory, expression of CD62L, was also examined. Both B6 and B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> splenic CD8 cells are predominantly CD62L<sup>low</sup> at birth. By day 28, the ratio of high:low cells had greatly increased in B6 animals but remained low in B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> mice. By day 56, this difference was even greater.

## IFN-γ secretion of CD8<sup>+</sup> CD44<sup>h</sup> vs CD8<sup>+</sup> CD44<sup>low</sup> cells from B6 and B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> mice

CD44<sup>high</sup> expression is a general marker for CD8 memory cells. However, it is not clear whether the CD44<sup>high</sup> cells in B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> mice are memory cells that have expanded as a result of Ag recognition or arisen as a result of homeostatic expansion. One of the functional phenotypes of CD8 memory vs naive cells is the ability of the former to rapidly secrete IFN-γ following activation (23). Therefore, we cultured B6 and B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> spleen cells with PMA and ionomycin and monitored the appearance of intracellular IFN-γ with time. Approximately 20% of all CD8 cells secrete this cytokine after 4 h from both B6 and B6.Kb<sup>h−/−/D<sup>b−/−</sup></sup> animals (Fig. 5a).
Analysis of the CD8<sup>+</sup>CD44<sup>low</sup> naive cells shows that <10% of the cells secrete IFN-γ at that time (Fig. 5b). However, CD8<sup>+</sup>CD44<sup>high</sup> memory cells secrete IFN-γ at a rapid rate. Approximately 70% of these cells from B6 mice secrete IFN-γ after 4 h (Fig. 5c). In contrast, CD8<sup>+</sup>CD44<sup>high</sup> cells from B6.K<sup>b</sup>D<sup>b</sup> mice show a small response at 1 h but this increased only modestly to 30% of cells after 4 h.

**Ability of CD8 cells to undergo homeostatic expansion in class Ia-deficient hosts**

It has been reported that under conditions of low SP T cell output from the thymus that peripheral SP T cells can compensate by homeostatic expansion to fill their compartment (24). Although the thymus from B6.K<sup>b</sup>D<sup>b</sup> mice has reduced numbers of SP CD8 T cells, it might therefore be expected that in the periphery these cells could expand to fill the CD8 compartment. Alternatively, class Ib-selected CD8 T cells might not be able to undergo homeostatic expansion and thus leave the CD8 compartment deficient in cell numbers. When we tested the ability of CFSE-labeled CD8<sup>+</sup> T cells from B6.K<sup>b</sup>D<sup>b</sup> mice to proliferate in unirradiated B6.K<sup>b</sup>D<sup>b</sup> animals, no proliferation was detected (data not shown). Although B6.K<sup>b</sup>D<sup>b</sup> CD8 T cells transferred into irradiated B6.K<sup>b</sup>D<sup>b</sup> mice did undergo proliferation (Fig. 6a), much of this was independent of class I molecules since proliferation was also observed in B6.K<sup>b</sup>D<sup>b</sup>β<sub>2</sub>-M<sup>−/−</sup> mice (Fig. 6b). However, some expansion of the B6.K<sup>b</sup>D<sup>b</sup> cells occurs on the non-class Ia background since 57.5 ± 2.5% proliferated in the B6.K<sup>b</sup>D<sup>b</sup> vs 26.5 ± 0.5% in the B6.K<sup>b</sup>D<sup>b</sup>β<sub>2</sub>-M<sup>−/−</sup> recipients. Even though most of the donor cells are CD44<sup>high</sup> that are thought to be able to undergo expansion independent of class I, most of the donor cells underwent a few rounds of divisions in either host.

**Percentage of CD8<sup>+</sup> T cells from B6 mice that show homeostatic proliferation in B6.K<sup>b</sup>D<sup>b</sup> mice**

Class Ia-deficient mice afford the opportunity to determine the ability of donor cells to undergo homeostatic proliferation when exposed to limited sets of Ags. Therefore, we analyzed the ability...
of B6 donor cells to undergo homeostatic proliferation in irradiated B6.Kb-Db−/− recipients. Since naive CD8 T cells in the periphery actively engage in interactions with self-MHC molecules that they were selected on, it was expected that the percentage of transferred cells that proliferate in recipients would be higher in B6.Kb-Db−/− than in B6.Kb−/− Db−/− mice. Transfer of B6.Thy1.1 CD8 cells into irradiated B6 recipients resulted in all donor cells dividing by day 5 (Fig. 7). In contrast, transfer of these cells into irradiated Kb−/− hosts resulted in 56 and 54% of cells not undergoing division, respectively, indicating that approximately half of CD8 T cells respond to either Kb or Db plus non-class Ia molecules to undergo homeostatic expansion. Transfer of B6 CD8 cells into B6.Kb-Db−/− hosts resulted in 68% of cells undergoing no cell divisions (Fig. 6, c vs d). A similar result is noted when these cells were transferred into B6.Kb−/− Db−/− β2-M− mice (Fig. 6, d–f), indicating that the percentage of non-class Ia-reactive CD8 T cells in normal B6 animals that undergo homeostatic expansion is very small. However, there was a difference between the ability of B6-Thy1.1 cells to proliferate in B6.Kb−/− Db−/− vs B6.Kb−/− Db−/− β2-M− mice in that a minor population (1–2%) of cells underwent more than four divisions in the former but not latter hosts (Fig. 6, c vs d, e, and f).

Discussion

Class I-deficient β2-M−/− or Tap-1−/− animals have greatly reduced numbers of CD8 T cells. This result may be expected since
present Tap-dependent peptides. In the case of B6.K$b$D$b$ mice that lack class Ia molecules, the deficiency in CD8 T cells likely reflects the reduced repertoire directed against class Ib molecules. This is evident in the thymus of these mice which contain ~15% of SP CD8 cells seen in B6 mice. It has been noted that SP cells in neonatal mice proliferate strongly and display a CD44$^{high}$ phenotype, reflecting the lymphopenic environment that they are exposed to (25, 26). We have compared the phenotype of splenic SP CD8 T cells in B6 and B6.K$b$D$b$ mice and observed that on day 1 both strains contained a predominance of cells that are CD44$^{high}$CD62L$^{low}$. However, by day 28 most B6 CD8 T cells had an immature phenotype while the mature phenotype persisted in B6.K$b$D$b$ mice. As a result, the total number of CD44$^{high}$CD8 T cells in both strains is similar, whereas B6.K$b$D$b$ mice are deficient in the naive cell subset. One explanation for the high number of CD44$^{high}$ cells in the B6.K$b$D$b$ mice is that they could be principally CD8αα T cells that are known to be CD44$^{high}$. However, we noted that ~90% of CD8 T cells from these mice are CD8αβ. It has been reported that thymic education of some class Ib-restricted CD8 T cells can occur on radiosensitive cells in the thymus, unlike that generally observed for class Ia-restricted cells (27, 28). Perhaps as a result of this, the SP CD8 thymocytes acquire a CD44$^{high}$ phenotype in situ (27). We examined mature SP CD8 thymocytes based on the levels of CD24 and Vβ expression and found that most of these cells are CD44$^{low}$. Thus, we conclude that the acquisition of the CD44$^{high}$ phenotype is a postthymic event similar to that seen in B6 animals.

CD44$^{high}$ is generally taken as a marker for CD8 memory. One characteristic of the memory phenotype is a rapid IFN-γ response following cell activation (23). Although this was noted in the CD8$^{+}$CD44$^{high}$ cells from B6 mice, CD8$^{+}$CD44$^{high}$ cells from B6.K$b$D$b$ mice did not show this rapid response. A rapid IFN-γ response is also characteristic of cells undergoing homeostatic expansion (13, 29). This suggests that the CD44$^{high}$ phenotype in the CD8 T cells from these mice does not necessarily represent a memory cell or a cell that has undergone homeostatic expansion. However, it is known that the CD8 T cell response to Ags restricted by M3 are quantitatively and qualitatively different from that observed against class Ia molecules and this altered phenotype may be representative of these type of cells (9, 10, 30). In any event, our current data indicate that most CD8 T cells that arise in an environment devoid of class Ia Ags and acquire a CD44$^{high}$ phenotype are different from conventional CD44$^{high}$ class Ia-restricted memory cells.

Recent data using the model of homeostatic expansion have shown that although there is a lymphoid compartment that regulates this expansion, it is not compartmentalized into CD4 and CD8 subsets (31). This issue is now being re-examined by the finding that TCR-transgenic T cells can expand in nonirradiated hosts that contain TCR-transgenic cells of another specificity (32, 33). We did not observe that transfer of B6 CD8 cells into CD8-deficient B6.K$b$D$b$ recipients allowed for expansion unless these hosts were irradiated before transfer (data not shown). Thus, it is not clear why the CD44$^{high}$ cells do not fill the CD8 compartment in these B6.K$b$D$b$ animals, since it has been reported that under conditions of low SP cell output from the thymus that peripheral SP T cells can compensate by homeostatic expansion (24) as well as results alluded to above suggesting that there is no single lymphoid compartment. One possibility is that cells specific for class Ib molecules do not get proper signaling to allow for complete expansion. This could be a result of low expression of most of these molecules, as has been documented for Qa-1 and M3 (2, 34). Transfer of CD8 T cells from B6.K$b$D$b$ mice into irradiated B6.K$b$D$b$ recipients results in the ability of some of these cells to undergo expansion, although the extent of cell divisions was much less than that observed of CD8 T cells responding to class Ia Ags. About one-half of the proliferation observed was seen in B6.K$b$D$b$β-M$^{-}$M$^{-}$ mice, indicating that it is independent of class I molecules. Another possibility, consistent with the recent data, is that CD8 T cell selection in the thymus, against at least some class Ib molecules, differs qualitatively from selection against class Ia molecules (27). This result may alter the ability of these cells to fill the CD8 compartment or explain the CD44$^{high}$ phenotype.

B6 CD8 T cells displayed extensive homeostatic expansion in irradiated syngeneic hosts, as expected (35, 36). Both K$b$D$b$ and D$b$-/- mice have an ~2-fold reduction in peripheral CD8 T cells (6). When we transferred B6 CD8 T cells into irradiated K$b$D$b$-/- and D$b$-/- hosts, we noted that although homeostatic proliferation occurred ~50% of donor cells did not undergo cell division in either host. This is consistent therefore with the decreased number of CD8 T cells and suggests that the CD8 repertoire in B6 mice is rather evenly distributed against both class Ia molecules. Transfer of B6 cells into B6.K$b$D$b$ mice also resulted in homeostatic expansion of donor cells. However, in these hosts only ~20% of cells divided and the extent of division was similar when these cells were transferred into B6.K$b$D$b$ vs B6.K$b$D$b$β-M$^{-}$M$^{-}$ mice. When comparing the difference in proliferation of B6 donor cells in B6.K$b$D$b$ vs B6.K$b$D$b$β-M$^{-}$M$^{-}$ recipients, it was only noted in a very small percentage of cells that underwent more than four divisions. This indicates that only a very small percentage of the TCR repertoire in B6 animals is non-class Ia restricted, based on the criteria of homeostatic expansion. It is also possible that at least some class Ia-selected T cells react against class Ib molecules as has been shown for the ability of the 2C T cell class Ia-reactive receptor to be selected in B6.K$b$D$b$ mice (11).

References


