

Development of CD8 α / α and CD8 α / β T Cells in Major Histocompatibility Complex Class I-deficient Mice

By Gobardhan Das and Charles A. Janeway, Jr.

From the Howard Hughes Medical Institute and Yale University Medical School, New Haven, Connecticut 06510

Summary

Peripheral CD8⁺ T cells mainly use CD8 α / β , and their development is mainly dependent on the major histocompatibility complex (MHC) class I proteins K^b and D^b in H-2^b mice. In this report, we have shown that the development of CD8 α / β TCR- α / β cells in lymphoid organs as well as in intestinal intraepithelial lymphocytes (iIELs) is dependent on the MHC class I K^b and D^b proteins. In contrast, TCR- α / β CD8 α / α cells are found mainly in iIELs, and their numbers are unaffected in K^bD^b double knockout mice. Most of the TCR- γ / δ cells in the iIELs also bear CD8 α / α , and they are also unaffected in K^bD^b -/- mice. In β 2-microglobulin (β 2m)-deficient mice, all of the TCR- α / β CD8 α / α and CD8 α / β T cells disappear, but TCR- γ / δ cells are unaffected by the absence of β 2m.

Key words: CD8 α / α T cells • CD8 α / β T cells • major histocompatibility complex class I • K^bD^b-deficient mice • β 2m-deficient mice

T cells that are CD8⁺ recognize antigens as peptides of 8–10 amino acids bound to MHC class I molecules. Deletion of either the K^b and D^b genes, the MHC class I-associated molecule β 2-microglobulin (β 2m), or the transporters associated with antigen processing (TAP) molecules severely reduces the numbers of CD8⁺ T cells in peripheral lymphoid organs (1–4). In the periphery, CD8⁺ T cells usually express CD8 α / β , which serves as a coreceptor for the MHC class I molecules during antigen recognition. By contrast, the majority of the CD8⁺ T cells residing in gut epithelia express CD8 α / α (5–11). Intestinal intraepithelial lymphocytes (iIELs) are composed of roughly equal numbers of TCR- α / β and TCR- γ / δ cells. Previous studies revealed that development of TCR- α / β cells among iIELs is β 2m and TAP dependent, whereas γ / δ T cells are dependent on neither β 2m nor TAP (12, 13). In this report, we examined the requirement of classical MHC class I K^b and D^b molecules in the development of CD8 α / α and CD8 α / β T cells. Our results demonstrate that CD8⁺ T cells in peripheral lymphoid organs like spleen and lymph node primarily bear CD8 α / β , whereas CD8⁺ T cells in iIELs mainly express CD8 α / α . In K^bD^b double knockout mice, most of the CD8 α / β -bearing TCR- α / β cells disappear in the periphery as well as in iIELs. CD8 α / α -bearing T cells are found mainly in iIELs, suggesting that their development is independent of K^bD^b antigens. In B6- β 2m knockout mice, CD8⁺ TCR- α / β cells disappear completely, but γ / δ T cells are unaffected even though some of them also bear a CD8 coreceptor. This suggests that the development of CD8 α / β TCR- α / β cells is mainly dependent

on K^b and D^b proteins, whereas CD8 α / α TCR- α / β cells are dependent on β 2m-associated MHC class Ib molecules and CD8 α / α TCR- γ / δ cells develop independently of MHC class I molecules altogether.

Materials and Methods

Mice and Antibodies. C57BL/6 (B6) and B6- β 2m-deficient mice were purchased from The Jackson Laboratory. K^bD^b double knockout mice were a gift from Dr. Hidde Ploegh (Dept. of Pathology, Harvard University School of Medicine, Boston, MA). Anti-TCR- α / β (H-57), anti-TCR- γ / δ (GL-3), and anti-CD8 β (Ly-3.2) antibodies were purchased from Pharmingen. Anti-CD8 α (53-6.7) was purchased from Sigma Chemical Co.

Isolation of Lymphocytes. For the isolation of lymphocytes from spleen and lymph node, spleens and lymph nodes were macerated individually using frosted glass slides. The resulting suspension was centrifuged on a Ficoll gradient at 900 *g* for 10 min. Cells at the interface were collected, and T cells were enriched by passing through a nylon wool column as described previously (14). iIELs were prepared as described previously (15). In brief, small intestines were harvested and washed by passing through PBS. Mesentery and Peyer's patches were carefully removed. The intestines were cut longitudinally and then in ~0.5-cm pieces. Intestinal pieces were agitated in 50 ml of extraction buffer (PBS, 3% FCS, and 10 mM EDTA) for 30 min at 37°C. This slurry was centrifuged at 2 *g* for 2 min to remove the aggregates. The cell suspension was layered on a discontinuous Percoll (Amersham Pharmacia Biotech) gradient. This gradient was then centrifuged at 900 *g* for 20 min. Cells at the interface of the 40/70% layer were collected and washed in staining buffer. Lymphocytes from the liver were prepared as described previously (16).

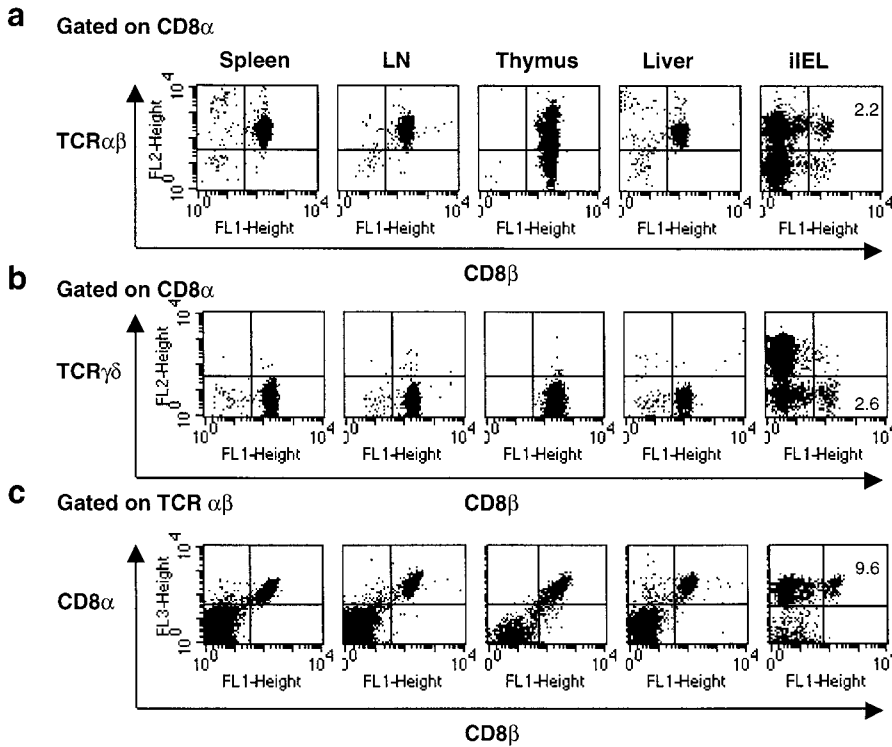


Figure 1. Organ distribution of CD8 α/α and CD8 α/β T cells. (a) In three-color staining, gating on CD8 α^+ T cells shows most of the CD8 α^+ cells bear TCR- α/β and CD8 β as well. (b) Only a few, or no, CD8 α^+ cells are found in TCR- γ/δ^+ in spleen, lymph node (LN), thymus, and liver, whereas a large portion of the cells are found in iIELs. (c) Gating on TCR- α/β shows that in iIELs only a small portion of the cells bear CD8 α/β and the majority of the cells are CD8 α^+ . In all other organs, all of the CD8 α^+ cells are positive for CD8 β also.

In brief, livers were macerated using stainless steel mesh and suspended in PBS, 3% FCS. They were loaded on a discontinuous Percoll gradient and centrifuged at 900 *g* for 20 min. Cells were collected from the 40/70% interface, washed, and used for further experiments.

FACS[®] Staining and Analysis. Cells were suspended in staining buffer (PBS, 3% FCS, 0.01% sodium azide) at a concentration of 10^7 cells/ml. 100 μ l of the suspension was incubated with directly conjugated antibodies for 30 min on ice. Cells were washed twice with staining buffer and fixed with 1% paraformaldehyde. Fluorescence intensities were measured with a FAC-Scan[™] (Becton Dickinson).

Results and Discussion

For positive selection, CD8⁺ T cells require MHC class I proteins, and in this process the CD8 molecules serve as coreceptor (17). In peripheral CD8⁺ T cells, the CD8 molecule is expressed as a membrane-bound heterodimeric protein consisting of α and β chains (18). However, certain CD8⁺ T cells express an alternate CD8 α/α homodimer. Thus, we examined the organ distribution of these unusual T cells. We found that most of the CD8⁺ T cells in spleen, lymph node, thymus, and liver express CD8 α/β , and they all consistently bear TCR- α/β . By contrast, in iIELs most of the T cells express CD8 α , but only a small portion of the T cells express CD8 β as well (Fig. 1 a). In spleen, lymph node, thymus, and liver, only a very small fraction of CD8 α^+ TCR- γ/δ cells were found. In contrast, in iIELs a large portion of the CD8 α/α^+ T cells were found to also be TCR- γ/δ^+ (Fig. 1 b). In three-color staining, gating on TCR- α/β revealed that in spleen, lymph node, thymus,

and liver, almost all of the CD8 α -bearing T cells express CD8 β , but in iIELs a large number of TCR- α/β cells express only CD8 α . Among these, only a fraction (9–11%) are CD8 β^+ (Fig. 1 c). On further analysis of the iIELs, it was found that among iIELs, TCR- α/β and TCR- γ/δ cells are present at almost equal numbers, and a majority of the cells express the CD8 cell surface molecule (Fig. 2 a). Gating on TCR- γ/δ cells among the iIELs revealed that most such cells express CD8 α and none express CD8 β (Fig. 2 b).

β 2m-associated MHC class I proteins are present on the cell surface at $\sim 10^5$ – 10^6 molecules per cell and are divided

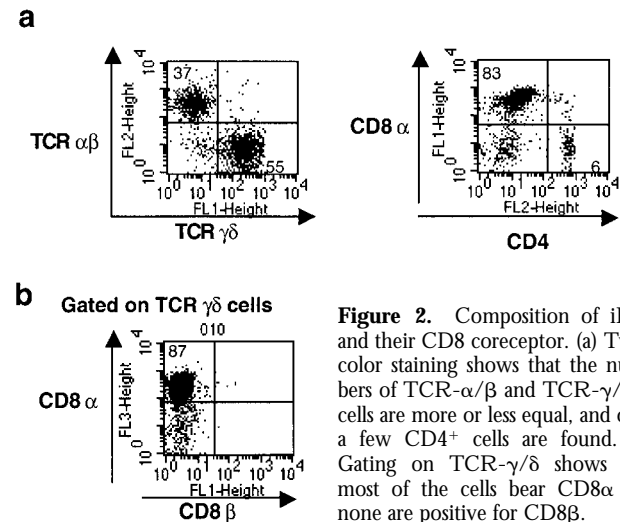


Figure 2. Composition of iIELs and their CD8 coreceptor. (a) Two-color staining shows that the numbers of TCR- α/β and TCR- γ/δ T cells are more or less equal, and only a few CD4⁺ cells are found. (b) Gating on TCR- γ/δ shows that most of the cells bear CD8 α but none are positive for CD8 β .

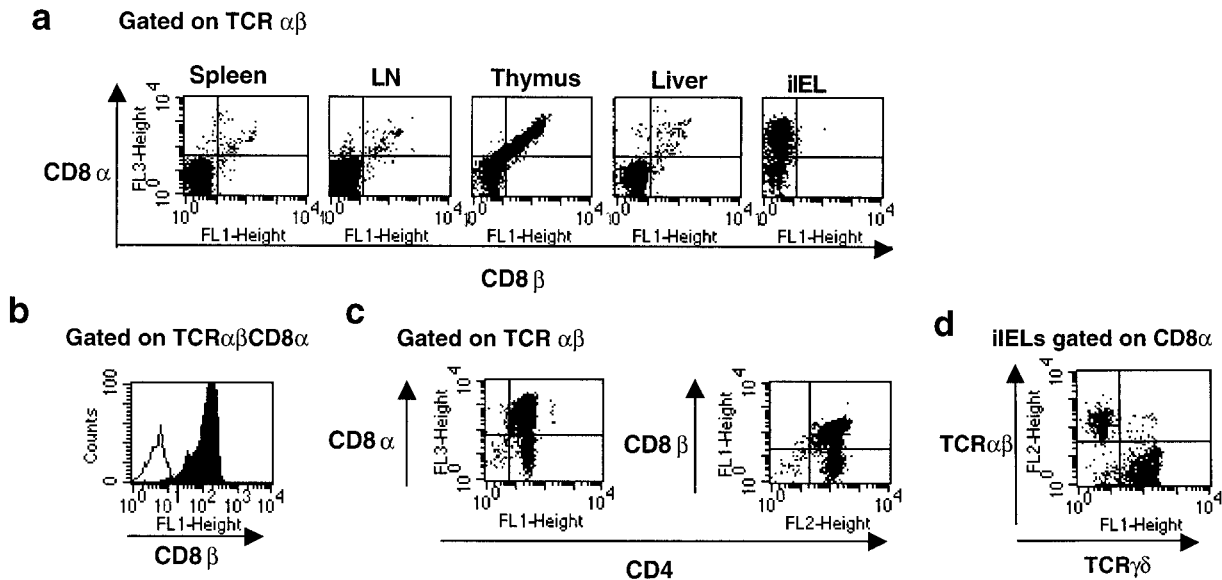


Figure 3. Composition of CD8 α/α and CD8 α/β T cells in different organs in K^bD^b double knockout mice. (a) Gating on TCR- α/β shows that in spleen, lymph node (LN), and liver, only a few CD8⁺ cells were found. In thymus and iIELs, a large number of CD8 cells were seen. They are CD8 α/β ⁺ in thymus and CD8 α ⁺CD8 β ⁻ in iIELs. In staining of thymus cells (b), gating on TCR- α/β CD8 α shows that all of the cells are CD8 β ⁺ as well. (c) Gating on TCR- α/β shows that only a few CD4⁻CD8 α ⁺ or CD4⁻CD8 β ⁺ cells are found. (d) In staining of iIELs gating on CD8 α , there were large numbers of TCR- α/β and TCR- γ/δ cells.

into two distinct categories. The classical MHC class I proteins are derived from the genes for K, D, and L, which in B6 mice are called K^b and D^b (there is no L molecule in the H-2^b haplotype). The nonclassical MHC class I proteins are called class Ib molecules, are the products of Qa, TL, and M regions, and are also coded by the non-MHC genes of the CD1 locus on chromosome 1.

To evaluate the type of MHC restriction of both CD8 α/α and CD8 α/β T cells, we examined the composition of these two groups of cells in K^bD^b double knockout mice. It was found that most of the CD8 α/β TCR- α/β cells disappeared in spleen, lymph node, and liver as well as in iIELs (Fig. 3 a). In iIELs, numbers of CD8 α ⁺ cells were

not affected. In the thymus, there were normal numbers of CD4⁺CD8⁺ double positive (DP) cells in K^bD^b double knockout mice. These are presumed to be of the TCR- α/β lineage. However, there were virtually no CD4⁻CD8⁺ single positive (SP) cells (Fig. 3 c), and all of the DP cells were found to use CD8 α/β (Fig. 3 b). Therefore, these DP cells in the thymus are uncommitted immature thymocytes that had not undergone intrathymic selection. Thus, CD8 α/α ⁺ T cells are mostly found in iIELs. This clearly indicates the potential for recognition of nonclassical MHC class Ib molecules by T cells bearing a TCR- α/β and the CD8 α/α homodimer.

To examine whether this group of CD8 α/α T cells re-

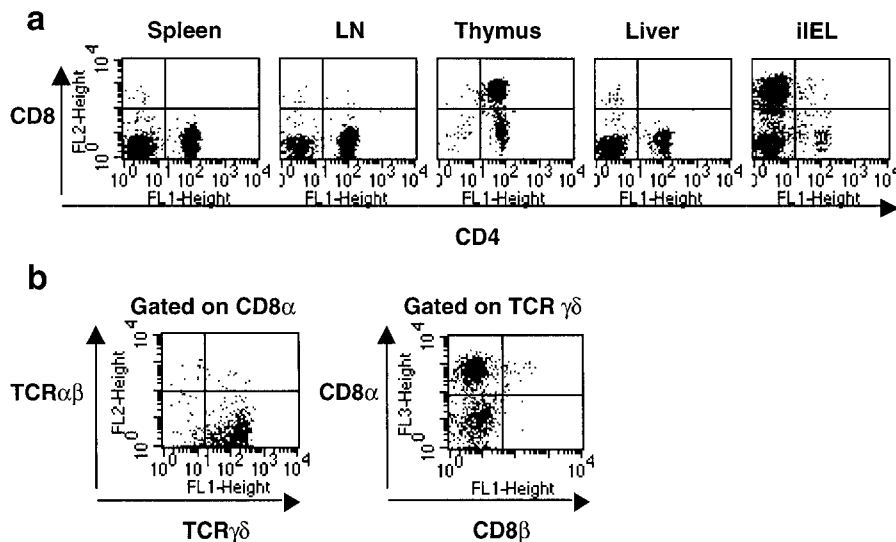


Figure 4. Evaluation of CD8⁺ T cells in $\beta 2m^{-/-}$ mice. (a) Spleen, lymph node (LN), and liver show absence of CD8⁺ cells; however, in thymus, vast numbers of CD4⁺CD8⁺ cells were found but no SP (CD8⁺) cells were seen. In iIELs, there are large numbers of CD8⁺ cells. (b) Gating on CD8 α shows that all of the cells in iIELs possess TCR- γ/δ but not TCR- α/β , and gating on TCR- γ/δ shows that most of the cells bear CD8 α but not CD8 β .

quired $\beta 2m$ -associated MHC class I or class Ib molecules, we analyzed the composition of CD8 α/α cells in $\beta 2m^{-/-}$ mice. It was found that CD8 $^{+}$ T cells are absent in spleen, lymph node, and liver. However, substantial numbers of CD8 α^{+} T cells were present in thymus and iIELs (Fig. 4 a). In thymus, all of the CD8 α^{+} T cells bear CD4 as well. No CD8 SP (CD4 $^{-}$ CD8 $^{+}$) cells were found in these mice. A careful analysis revealed that all CD8 α^{+} T cells in iIELs possess a γ/δ TCR (Fig. 4 b). Thus, the development of the vast majority of the TCR- α/β CD8 α/β cells requires contact with K b or D b molecules. By contrast, CD8 α/α TCR- α/β cells depend on $\beta 2m$ -associated nonclassical

MHC class Ib molecules. The development of CD8 α^{+} TCR- γ/δ cells in iIELs either does not require MHC class I for their positive selection or they are restricted to $\beta 2m$ -independent MHC class I molecules. The existence of $\beta 2m$ -independent MHC class I molecules is as yet an open question. Recently, a group of stress-induced $\beta 2m$ -independent MHC class I molecules was reported in humans, but the homologous genes are not found in mice (19). Thus, the diversity of a $\beta 2m$ -independent MHC class I molecule in the gut is a worthy goal for future research on the development of CD8 α^{+} γ/δ T cells.

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Address correspondence to Charles A. Janeway, Jr., LH-416, Howard Hughes Medical Institute and Yale University School of Medicine, 310 Cedar St., New Haven, CT 06510. Phone: 203-785-2793; Fax: 203-737-1765; E-mail: charles.janeway@yale.edu

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References

- Vugmeyster, Y., R. Glas, B. Perarnau, F.L. Lemonnier, H. Eisen, and H. Ploegh. 1998. Major histocompatibility complex (MHC) class I K b D b $-/-$ deficient mice possess functional CD8 $^{+}$ T cells and natural killer cells. *Proc. Natl. Acad. Sci. USA.* 95:12492-12497.
- Zijlstra, M., M. Bix, N.E. Semister, J.M. Loring, D.H. Raulet, and R. Jaenisch. 1990. $\beta 2$ -microglobulin deficient mice lack CD4 $^{-}$ CD8 $^{+}$ cytolytic T cells. *Nature.* 344:742-746.
- Raulet, D.H. 1994. MHC class I-deficient mice. *Adv. Immunol.* 55:381-421.
- Van Kaer, L., P.G. Ashton-Rickard, H.L. Ploegh, and S. Tonegawa. 1992. TAP1 mutant mice are deficient in antigen presentation, surface class I molecules and CD4 $^{-}$ CD8 $^{+}$ T cells. *Cell.* 71:1205-1214.
- Lefrancois, L. 1991. Phenotypic complexity of intraepithelial lymphocytes of the small intestine. *J. Immunol.* 147:1746-1751.
- Guy-Grand, D., N. Cerf-Bensussan, B. Malissen, M. Malassis-Seris, C. Brittet, and P. Vassalli. 1991. Two gut intraepithelial CD8 $^{+}$ lymphocyte populations with different T cell receptors: a role for the gut epithelium in T cell differentiation. *J. Exp. Med.* 173:471-481.
- Poussier, P., P. Edouard, C. Lee, M. Binnie, and M. Julius. 1992. Thymus-independent development and negative selection of T cells expressing T cell receptor α/β in the intestinal epithelium: evidence for distinct circulation patterns of gut- and thymus-derived T lymphocytes. *J. Exp. Med.* 176:187-199.
- Rocha, B., P. Vassalli, and D. Guy-Grand. The extrathymic T-cell development pathway. *Immunol. Today.* 13:449-454.
- Lefrancois, L. 1991. Extrathymic differentiation of intraepithelial lymphocytes: generation of a separate and unique T-cell repertoire? *Immunol. Today.* 12:436-438.
- Hass, W., P. Pereira, and S. Tonegawa. 1993. γ/δ cells. *Annu. Rev. Immunol.* 11:637-685.
- Rocha, B., P. Vassalli, and D. Guy-Grand. 1991. The V β repertoire of mouse gut homodimeric α CD8 $^{+}$ intraepithelial T cell receptor α/β^{+} lymphocytes reveals a major extrathymic pathway of T cell differentiation. *J. Exp. Med.* 173:483-486.
- Fujiura, Y., M. Kawaguchi, Y. Kondo, S. Obana, H. Yamamoto, M. Nanno, and H. Ishikawa. 1996. Development of CD8 $\alpha\alpha^{+}$ intestinal intraepithelial T cells in $\beta 2$ -microglobulin and/or TAP-1 deficient mice. *J. Immunol.* 156:2710-2715.
- Sydora, B.C., L. Brossay, A. Hargenbaugh, M. Kronenberg, and H. Cheroutre. 1996. TAP-independent selection of CD8 $^{+}$ intestinal intraepithelial lymphocytes. *J. Immunol.* 156:4209-4216.
- Das, G., H. Vorah, J.N. Agrwala, and G.C. Mishra. 1999. Apoptosis of Th1-like cells in experimental tuberculosis (TB). *Clin. Exp. Immunol.* 115:324-328.
- Van der Heijden, P.J., and W. Stok. 1987. Improved procedure for the isolation of functionally active lymphoid cells from the murine intestine. *J. Immunol. Methods.* 103:161-167.
- Ohteki, T., and H.R. MacDonald. 1994. Major histocompatibility complex class I related molecules control the development of CD4 $^{+}$ CD8 $^{-}$ and CD4 $^{-}$ CD8 $^{-}$ subsets of natural killer 1.1 $^{+}$ T cell receptor- α/β^{+} cells in the liver of mice. *J. Exp. Med.* 180:699-704.
- Sprent, J. 1993. T lymphocyte and the thymus. In *Fundamental Immunology*, 3rd ed. Raven Press, Ltd., New York. 75-109.
- Carayannopoulos, L., and D.J. Capra. 1993. Immunoglobulins: structure and function. In *Fundamental Immunology*, 3rd ed. Raven Press, Ltd., New York. 283-314.
- Groh, V., A. Steinle, S. Bauer, and T. Spies. 1998. Recognition of stress induced MHC molecules by intestinal epithelium $\gamma\delta$ T cells. *Science.* 279:1737-1740.