Brief Definitive Report

Development of CD8α/α and CD8α/β T Cells in Major Histocompatibility Complex Class I-deficient Mice

By Gobardhan Das and Charles A. Janeway, Jr.

Summary

Peripheral CD8+ T cells mainly use CD8α/β, and their development is mainly dependent on the major histocompatibility complex (MHC) class I proteins Kβ and Dβ in H-2b mice. In this report, we have shown that the development of CD8α/β TCR-α/β cells in lymphoid organs as well as in intestinal intraepithelial lymphocytes (iIELs) is dependent on the MHC class I Kβ and Dβ proteins. In contrast, TCR-α/β CD8α/α cells are found mainly in iIELs, and their numbers are unaffected in KβDβ double knockout mice. Most of the TCR-γδ cells in the iIELs also bear CD8α/α, and they are also unaffected in KβDβ−/− mice. In β2-microglobulin (β2m)-deficient mice, all of the TCR-α/β CD8α/α and CD8α/β T cells disappear, but TCR-γδ cells are unaffected by the absence of β2m.

Key words: CD8α/α T cells • CD8α/β T cells • major histocompatibility complex class I • KβDβ-deficient mice • β2m-deficient mice

Materials and Methods

Mice and Antibodies. C57BL/6 (B6) and B6-β2m-deficient mice were purchased from The Jackson Laboratory. KβDβ 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-γδ (GL-3), and anti-CD8β (Ly-3.2) antibodies were purchased from PharMingen. Anti-CD8α (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. Mesentry and Peyer's patches were carefully removed. The intestines were cut longitudinally and then into 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. The 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).
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 10⁷ cells/ml. 100 μl 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 FACScan™ (Becton Dickinson).

**Results and Discussion**

For positive selection, CD8α 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 CD8α⁺ T cells express an alternate CD8α/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 CD8α/β, and they all consistently bear TCR-α/β. By contrast, in iIELs most of the T cells express CD8α, but only a small portion of the T cells express CD8β as well (Fig. 1 a). In spleen, lymph node, thymus, and liver, only a very small fraction of CD8α⁺ TCR-γδ T cells were found. In contrast, in iIELs a large portion of the CD8α⁺ T cells were found to also be TCR-γδ⁺ (Fig. 1 b). In three-color staining, gating on TCR-α/β reveals that in spleen, lymph node, thymus, and liver, almost all of the CD8α-bearer T cells express CD8β, but in iIELs a large number of TCR-α/β cells express only CD8α. Among these, only a fraction (9-11%) are CD8β⁺ (Fig. 1 c). On further analysis of the iIELs, it was found that among iIELs, TCR-α/β and TCR-γδ 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-γδ cells among the iIELs revealed that most such cells express CD8α and none express CD8β (Fig. 2 b).

β2m-associated MHC class I proteins are present on the cell surface at ~10⁵–10⁶ molecules per cell and are divided...
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 Kb and Db (there is no L molecule in the H-2b 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 CD8α/α and CD8α/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 CD8α/β TCR-α/β cells disappeared in spleen, lymph node, and liver as well as in iIELs (Fig. 3 a). In iIELs, numbers of CD8α+ 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−CD8α+ or CD4−CD8β+ cells found. In staining of iIELs gating on CD8α, there were large numbers of TCR-α/β and TCR-γ/δ cells.

Figure 3. Composition of CD8α/α and CD8α/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 CD8α/β in thymus and CD8α+CD8β- in iIELs. In staining of thymus cells (b), gating on TCR-α/β CD8α shows that all of the cells are CD8β+ as well. (c) Gating on TCR-α/β shows that only a few CD4−CD8α+ or CD4−CD8β+ cells are found. (d) In staining of iIELs gating on CD8α, there were large numbers of TCR-α/β and TCR-γ/δ cells.

Figure 4. Evaluation of CD8+ T cells in β2m−/− 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 CD8α 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 CD8α but not CD8β.
required β2m-associated MHC class I or class Ib molecules, we analyzed the composition of CD8α/α cells in β2m−/− mice. It was found that CD8+ T cells are absent in spleen, lymph node, and liver. However, substantial numbers of CD8α− T cells were present in thymus and iIELs (Fig. 4 a). In thymus, all of the CD8α− T cells bear CD4 as well. No CD8 SP (CD4−CD8+) cells were found in these mice. A careful analysis revealed that all CD8α− T cells in iIELs possess a γ/δ TCR (Fig. 4 b). Thus, the development of the vast majority of the TCR-α/β CD8α/β cells requires contact with Kb or Db molecules. By contrast, CD8α/α TCR-α/β cells depend on β2m-associated nonclassical MHC class Ib molecules. The development of CD8α+ TCR-γ/δ cells in iIELs either does not require MHC class I for their positive selection or they are restricted to β2m-independent MHC class I molecules. The existence of β2m-independent MHC class I molecules is as yet an open question. Recently, a group of stress-induced β2m-independent MHC class I molecules was reported in humans, but the homologous genes are not found in mice (19). Thus, the diversity of a β2m-independent MHC class I molecule in the gut is a worthy goal for future research on the development of CD8α+ γ/δ T cells.

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