

Qa-2–dependent Selection of CD8 α / α T Cell Receptor α / β ⁺ Cells in Murine Intestinal Intraepithelial Lymphocytes

By Gobardhan Das,^{*} Dina S. Gould,[‡] Mathew M. Augustine,^{*} Gladis Fragoso,[¶] Edda Scitto,[¶] Iwona Stroynowski,^{||} Luc Van Kaer,[§] Danny J. Schust,[‡] Hidde Ploegh,[‡] and Charles A. Janeway, Jr.^{*}

From the ^{*}Section of Immunobiology, Yale University School of Medicine, and the Howard Hughes Medical Institute, New Haven, Connecticut 06520-8011; the [‡]Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115; the [§]Department of Microbiology and Immunology and The Howard Hughes Medical Institute, Vanderbilt University School of Medicine, Nashville, Tennessee 37232; the ^{||}Center for Immunology and the Department of Microbiology and the Department of Internal Medicine, Southwestern Medical Center, Dallas, Texas 75235-9093; and the [¶]Department of Immunology, Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Mexico D.F. 04510

Abstract

Murine intestinal intraepithelial lymphocytes (iIELs) are made up of a heterogeneous mix of T cells with unique phenotypes. Whereas CD8⁺ T cells in peripheral lymphoid organs use CD8 α / β and are selected on MHC class Ia molecules, a majority of iIELs use CD8 α / α . Here, we report that the presence of CD8 α / α TCR- α / β cells in iIELs is independent of classical MHC class I molecules K^b and D^b, as illustrated by their presence in K^b/D^b double-knockout mice and in mice lacking a nonclassical MHC class I molecule, CD1d. Most strikingly, their presence is decreased by ~70% in mice lacking transporter associated with antigen processing (TAP). The TAP-dependent nonclassical MHC class I molecule Qa-2 is strongly implicated in the presence of these cells, as inferred from the low numbers of CD8 α / α TCR- α / β T cells in mice deficient in Qa-2 genes. Second, a Qa-2-transgenic mouse made in a Qa-2⁻ strain showed an increase in the numbers of CD8 α / α cells among its iIELs. Thus, the presence of CD8 α / α TCR- α / β cells in iIELs is mainly dependent on the nonclassical MHC class I molecule Qa-2.

Key words: CD8 α / α TCR- α / β cells • MHC class I-deficient mice • Qa-2-transgenic mice • Qa-2-deficient mice • intestinal intraepithelial lymphocyte

Introduction

In contrast to conventional thymus-derived α / β TCR cells, intestinal intraepithelial lymphocytes (iIELs) are a distinct population having several unique features. The vast majority of such cells are CD8⁺, the frequency of α / β and γ / δ T cells is roughly equal, and most γ / δ T cells express a CD8 α / α homodimer and may develop extrathymically (1–8). TCR- α / β cells in the iIEL compartment are generally heterogeneous in terms of their expression of CD8 α / α and CD8 α / β . CD8 α / β cells are reported to govern conventional immune responses against pathogens and environmental antigens (9–14). Information regarding the CD8 α / α

TCR- α / β subset of iIELs is still lacking information about the MHC molecules that they recognize and that present antigen to them. They appear to be a resident subset, do not participate in antigen-specific immune responses, and may play a role(s) in localized immune regulation (15, 16). Mice aged 6–8 wk and raised in specific pathogen-free conditions contain an equal number of α / β and γ / δ T cells among their iIELs, which are primarily, but not solely, CD8 α / α (17). Previously, it had been shown that the classical MHC class I molecules, K^b and D^b, are required for the presence of CD8 α / β TCR- α / β cells in peripheral lymphoid organs as well as in iIELs. On the other hand, CD8 α / α TCR- α / β cells were found only in intestinal epithelium and do not require the classical MHC class I molecules K^b and D^b (17–19). However, the presence of such cells is dependent on β_2 -microglobulin (β_2 m), suggesting a requirement for an MHC-related protein (20).

Address correspondence to Charles A. Janeway, Jr., Section of Immunobiology, P.O. Box 208011, LH416, 310 Cedar St., New Haven, CT 06520-8011. Phone: 203-785-2793; Fax: 203-737-1765; E-mail: charles.janeway@yale.edu

Here, we report that while the maintenance of CD8⁺ TCR- α/β iIELs is dependent on β_2m and transporter associated with antigen processing (TAP) function, the absence of the K^b and D^b molecules does not alter the development or maintenance of such cells. This observation allowed us to study the involvement of TAP-dependent nonclassical MHC molecules on the maintenance of TCR- α/β cells among the iIELs. All of the MHC class I molecules and the well studied, nonclassical MHC class Ib molecules Qa-2 proteins are known to be expressed in a TAP-dependent fashion (21). These proteins are encoded by four genes (Q6, Q7, Q8, and Q9) in C57BL/6 mice (22), contributing to a Qa-2^{high} phenotype (23). BALB/cJ mice carry only two functional Qa-2 genes (Q6 and Q7; reference 24) and express Qa-2 at an intermediate level (Qa-2^{dull}; reference 25). We find that BALB/cJ mice have a corresponding decrease in the percentage of CD8 α/α TCR- α/β cells in their iIEL compartments. BALB/cByJ and C3H/HeJ mice, which lack functional Qa-2 genes altogether and are therefore deficient in Qa-2 expression (23, 25, 26), have a severe deficit in the number of CD8 α/α TCR- α/β ⁺ iIELs. To further confirm the apparent importance of Qa-2 on the presence of CD8 α/α TCR- α/β T cells in iIELs, we examined their abundance in Qa-2-transgenic mice. Indeed, the Qa-2-transgenic mice produce higher numbers of CD8 α/α TCR- α/β iIELs. Thus, Qa-2 by itself can account for most of the TAP-dependent presence of CD8 α/α TCR- α/β cells among iIELs.

Materials and Methods

Mice and Antibodies. C57BL/6, BALB/c, BALB/cByJ, C3H/HeJ, B6C3F1/J, CByB6F1/J, and B6- $\beta_2m^{-/-}$ mice were purchased from The Jackson Laboratory. TAP^{-/-} mice, which had been backcrossed with C57BL/6 mice for 12 generations, were a gift from Dr. K.A. Hogquist (University of Minnesota, Minneapolis, MN). The K^bD^b^{-/-} mice were described previously (27).

CD1^{-/-}TAP^{-/-} mice were generated by an F1 brother and sister mating of CD1^{-/-} and TAP^{-/-} mice. CD1d^{-/-} mice were described previously (28), as were Qa-2-transgenic mice (29). In brief, these mice were produced in (C57BL/6 \times BALB/AnN) F1 and backcrossed to BALB/cAnN for 10 generations. 6–8-wk-old mice, irrespective of their sex, were used throughout the study. Mice were maintained in a pathogen-free colony and fed sterile food prepared at the Yale Animal Research Center. Anti-TCR- α/β (H-57), anti-TCR- γ/δ (GL-3), anti-CD8 β (Ly-3.2), and anti-Qa-2 (1-1-2), anti-V β 2 (B20), -V β 3 (KJ25), -V β 4 (KT4), -V β 5.1,5.2 (MR9-4), -V β 6 (RR4-7), -V β 7 (TR310), -V β 8.1,8.1 (MR5-2), -V β 9 (MR10-2), -V β 10b (B21.5), -V β 11 (RR3-15), -V β 12 (MR12-3), -V β 14 (14-2), and anti-CD1D (1B1) antibodies were purchased from PharMingen. Anti-CD8 α (53-6.7) was purchased from Sigma-Aldrich. Anti-MHC class I antibody (K^b and D^b; HB-51) was purified from the culture supernatant.

Preparation of iIELs. iIELs were prepared as described earlier (30) with a minor modification. In brief, small intestines were harvested and washed by swirling in PBS. Mesentery and Peyer's patches were carefully removed. The intestines were cut longitudinally and then into \sim 0.5-cm pieces. Intestinal pieces were agitated in 25 ml of extraction buffer (PBS, 3% FCS, 1 mM dithiothreitol, 1 mM EDTA) for 30 min at 37°C. This slurry was passed through a loosely packed nylon wool column to remove the aggregates. The follow-through was layered on a discontinuous Percoll gradient (Amersham Pharmacia Biotech). 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.

FACS[®] Staining and Analysis. Cells were suspended in staining buffer at a concentration of 10⁷ cells/ml. 100 μ l of suspension was incubated either with directly conjugated antibodies or biotinylated antibodies for 30 min on ice. For the latter, streptavidin-PE was used as the secondary reagent for an additional incubation of 30 min on ice. Cells were washed twice with staining buffer and fixed with 1% paraformaldehyde. Fluorescence intensities were measured with FACScan[™] (Becton Dickinson). Cells used for sorting were stained in Click's Earls Hanks amino acids, 5% FCS media. Cells were sorted by using a FACStar^{PLUS}[™] (Becton Dickinson).

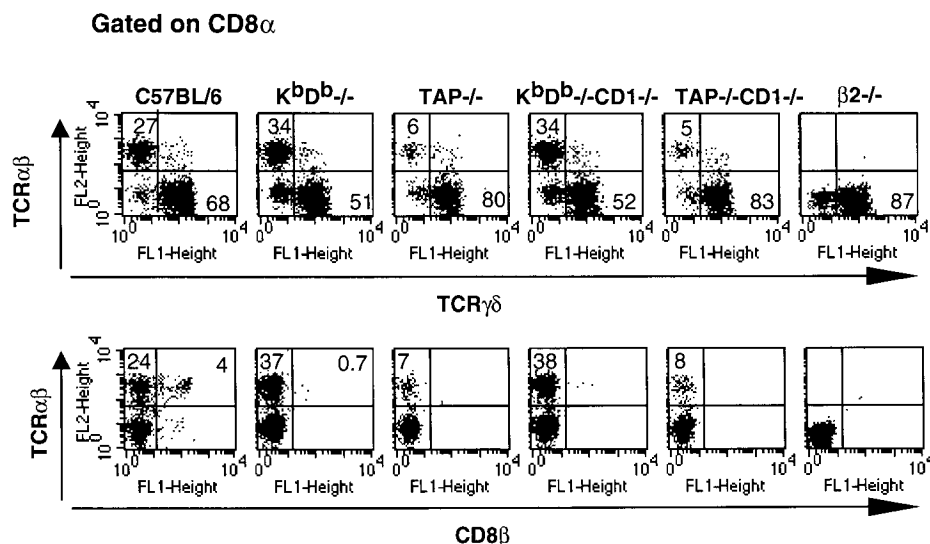


Figure 1. CD8 α/α TCR- α/β iIELs are present in K^b/D^b double-knock-out mice but absent in TAP^{-/-} and/or $\beta_2m^{-/-}$ mice. Comparison of iIELs from C57BL/6, K^bD^b^{-/-}, and K^bD^b^{-/-}CD1^{-/-} mice showed almost equal numbers of CD8 α/α TCR- α/β cells, whereas TAP^{-/-}, TAP^{-/-}CD1^{-/-}, and $\beta_2m^{-/-}$ mice had only a few of these cells. TCR- γ/δ cell numbers are not affected in any of these mice. The data shown represents six independent experiments.

Results and Discussion

Mice deficient in MHC class I expression lack peripheral CD8 α/β T cells (31, 32). To test the fate of intestinal T cells, we analyzed the composition of iIELs in $\beta_2m^{-/-}$ mice. Compared with wild-type animals, TCR- α/β iIELs in these mice disappear almost completely. There was no significant difference in the number of γ/δ T cells among the iIELs of $\beta_2m^{-/-}$ mice (Fig. 1 and reference 18). Hence, γ/δ T cells in the gut wall develop independently of MHC class I molecules in $\beta_2m^{-/-}$ animals. In contrast, the development of TCR- α/β iIELs requires the presence of β_2m -dependent MHC class I molecules. As β_2m is involved in the assembly of both classical (Ia) and nonclassical (Ib) MHC class I products, the above experiment does not differentiate between the roles of MHC class Ia and Ib molecules in the development and persistence of CD8 TCR- α/β iIELs. The availability of $K^bD^b^{-/-}$ mice (27) allowed us to separate genetically the functional contribution of MHC class Ib molecules from those of MHC class Ia molecules.

In H-2^b mice, most of the thymus-derived peripheral CD8 T cells develop on the products of either the K^b or D^b gene; the deletion of these two genes severely reduces the number of CD8 T cells in peripheral lymphoid organs (27). In contrast, the composition of iIELs in $K^bD^b^{-/-}$ mice showed no detectable reduction in CD8 α/α TCR- α/β cell numbers (Fig. 1).

In peripheral lymphoid organs, most of the CD8 T cells bear the CD8 α/β heterodimer, whereas TCR- α/β cells in iIELs bear either the CD8 α/β or CD8 α/α homodimer.

Table I. $V\beta$ Chain Usage by FACS[®] in iIELs of C57BL/6 and $K^bD^b^{-/-}CD1^{-/-}$ Mice

$V\beta$ usage	C57BL/6	$K^bD^b^{-/-}CD1^{-/-}$
2	5.8 \pm 0.13	4.21 \pm 0.3
3	5.08 \pm 1.4	4.5 \pm 0.25
4	3.07 \pm 1.62	4.04 \pm 1.08
5	13.04 \pm 3.8	11.5 \pm 6
6	5.62 \pm 0.6	5.4 \pm 1.4
7	2.7 \pm 0.24	1.5 \pm 0.3
8.12	16.44 \pm 3.5	14.7 \pm 3.3
8.3	8 \pm 1.5	8 \pm 0.95
9	1.44 \pm 0.12	1.53 \pm 0.25
10	4.03 \pm 0.9	3.8 \pm 1.14
11	11.33 \pm 1.5	11.32 \pm 1.95
12	3.43 \pm 0.3	2.76 \pm 0.3
13	4.1 \pm 1	4.64 \pm 1
14	3.97 \pm 0.9	2.4 \pm 0.20
17	1.57 \pm 0.15	1.98 \pm 0.3

In H-2^b mice, TCR- α/β iIELs do not require K^b and D^b for their development and maintenance, nor are they the result of a clonally expanded population. As shown, there was no remarkable skewing of TCR usage.

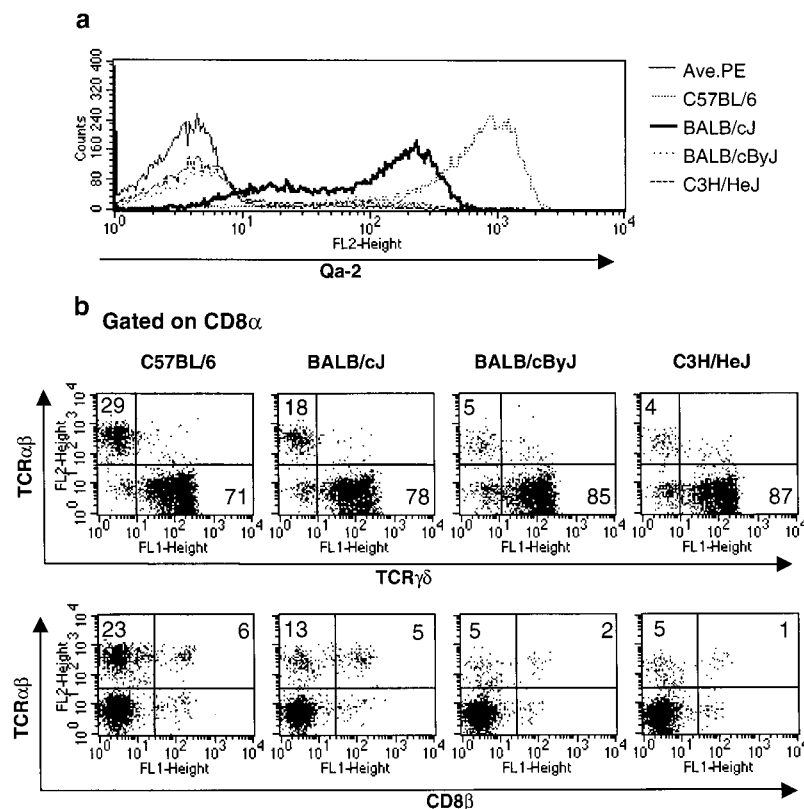


Figure 2. Qa-2 is implicated in the presence of CD8 α/α TCR- α/β iIELs. (a) Expression of Qa-2 on iIELs was measured by FACS[®]. BALB/cByJ and C3H/HeJ mice were deficient in Qa-2 protein, BALB/cJ mice show an intermediate level of Qa-2 expression, and C57BL/6 mice show the highest level of Qa-2 expression. (b) The level of CD8 α/α TCR- α/β iIELs was proportional to the level of Qa-2 expression. The highest numbers of these cells appear in C57BL/6 mice, an intermediate number in BALB/cJ mice, and the lowest numbers in BALB/cByJ and C3H/HeJ mice, which lack Qa-2 genes altogether. Data shown is representative of four independent experiments. Ave. PE, streptavidin R-PE.

Previously, it was reported that the appearance of CD8 α / β cells among iIELs increases with age (33) and environmental pathogens (9–14, 34). Recently, it was found that the presence of CD8 α / β T cells in peripheral lymphoid organs is mainly dependent on K^b and D^b molecules (17). Thus, we analyzed the content of α/β TCR iIELs in relatively young mice, where we had previously detected mainly CD8 α / α cells (17). The population of CD8 α / α TCR- α/β cells in the K^bD^b^{-/-} mouse might bear a diverse repertoire or might result from expansion of a clonal population of T cells. To address this question, we examined the usage of V β genes by the CD8 α / α TCR- α/β iIELs from several individual mice by FACS[®] staining. We observed representation of all commonly used V β chains without marked skewing of V β usage. This implies the presence of a diverse T cell receptor repertoire in CD8 α / α TCR- α/β cells (Table I) and argues against selective clonal expansion of iIELs in K^bD^b^{-/-} animals.

Because $\beta_2m^{-/-}$ mice are highly deficient in CD8 α / α TCR- α/β iIELs and mice lacking the classical MHC class Ia molecules have normal numbers of these cells, it follows that the development of iIELs is dependent on MHC class Ib molecules. To determine if selection of CD8 α / α TCR- α/β iIELs involves a TAP-dependent MHC class Ib product, we performed FACS[®] analysis on iIELs from TAP1^{-/-}

mice. iIELs from TAP1^{-/-} mice contain only one-third of the TCR- α/β cells found in wild-type mice and thus demonstrate a requirement for the MHC class Ib products that are TAP dependent (Fig. 1). This observation supports previously reported TAP-dependent selection and maintenance of TCR- α/β iIELs (20, 35). Considering data from both TAP^{-/-} and K^bD^b^{-/-} mice, we conclude that the development and maintenance of CD8 α / α TCR- α/β iIELs is dependent, at least partially, on one or more TAP-dependent nonclassical MHC class Ib molecule(s).

Several reports indicate that intestinal epithelial cells express a restricted set of MHC class Ib molecules, including CD1d (36). To investigate the possible involvement of CD1d molecules in the selection and maintenance of CD8 TCR- α/β iIELs, we performed FACS[®] analysis of iIEL populations in CD1d^{-/-} and TAP^{-/-}CD1d^{-/-} mice, and we found no difference in TCR- α/β T cell content when compared with normal or TAP^{-/-} mice, respectively (Fig. 1). Thus, CD1d does not significantly contribute to the development of α/β T cells in the iIEL compartment. This is consistent with a CD8 α / β T cell phenotype independent of TAP and CD1d expression (37).

Among the well characterized nonclassical MHC class Ib molecules, Qa-2 has been shown to be expressed in a TAP-dependent fashion (21). In contrast to most of the nonclassical MHC class Ib molecules, Qa-2 binds a wide

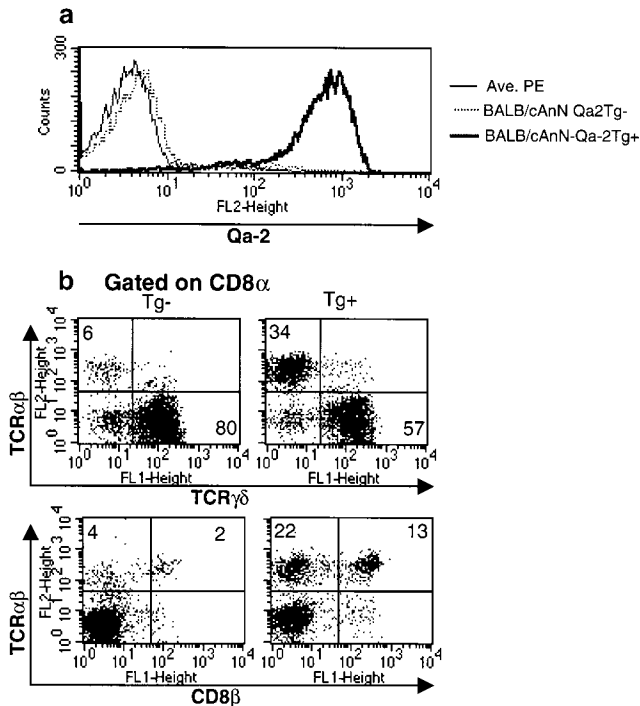


Figure 3. CD8 α / β TCR- α/β iIELs in Qa-2-transgenic mice. iIELs were separated from Qa-2-transgenic and nontransgenic mice. (a) Expression of Qa-2 is high in transgenic mice and virtually absent in BALB/cAnN mice. (b) FACS[®] analysis shows that mice that express Qa-2 from a transgene (Tg) have elevated numbers of CD8 α / α TCR- α/β cells that are comparable to the numbers found in wild-type mice (compare to Fig. 1). Results are compiled from four individual transgenic and three individual control mice analyzed on three separate days.

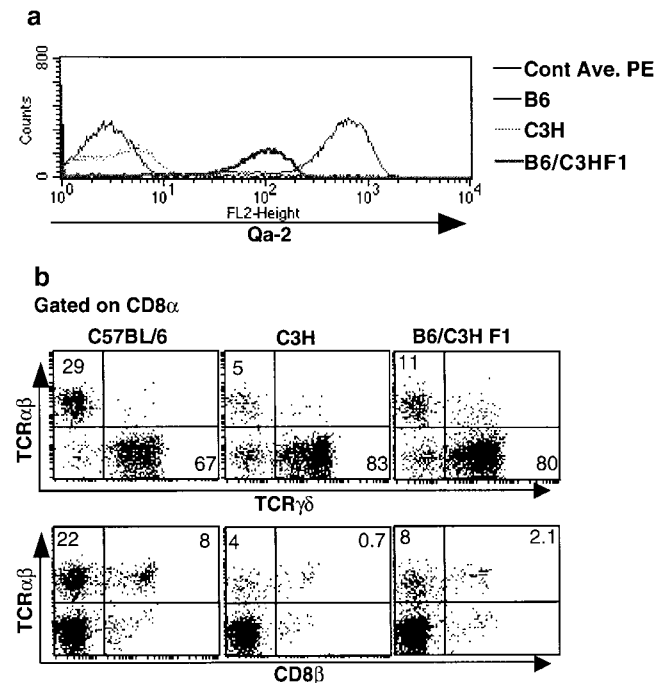


Figure 4. Intermediate level of Qa-2-expressing F1 mice from Qa-2⁺ and Qa-2⁻ parents show intermediate numbers of both CD8 α / α TCR- α/β and CD8 α / β TCR- α/β iIELs. iIELs were separated from Qa-2⁺ C57BL/6, Qa-2⁻ C3H, and Qa-2^{+/-} F1 mice and stained for Qa-2, CD8 α , CD8 β , TCR- α/β , and TCR- $\gamma\delta$. (a) iIELs from F1 mice showed an intermediate level of Qa-2 expression. (b) F1 mice derived from Qa-2⁺ and Qa-2⁻ parents showed an intermediate number of CD8 α / α TCR- α/β and CD8 α / β TCR- α/β cells. Cont. Ave. PE, control streptavidin R-PE.

variety of endogenous peptides (38). Therefore, it was expected that T cells that recognize Qa-2 would use a wide repertoire of TCRs. In fact, when we analyzed the TCRs used by the CD8 α / α TCR- α / β cells in K^bD^b^{-/-} mice, we found that these cells use a diverse set of TCR β chains similar to those in C57BL/6 mice (Table I).

Qa-2 proteins are expressed in various cell types, including epithelial cell lines (39). Their expression status in enterocytes is currently unknown, although Qa-2 transcription was detected in gut tissue (Chiang, E.Y. and I. Stroynowski, unpublished data). Qa-2-deficient mice are susceptible to *Taenia crassiceps* infection, whereas the presence of Qa-2 in host mice confers resistance to this parasite (29). These characteristics suggest that Qa-2 may play a role in the selection and/or maintenance of iIELs. We therefore examined the iIEL population present in two closely related strains of BALB/c mice, BALB/cJ (Qa-2^{dull}) and BALB/cByJ (Qa-2^{null}). These two strains were genetically separated around 60 years ago and differ at very few loci (40), including one known mutation of MHC class I genes. This mutation involved internal deletion of functional Qa-2 genes, which led to silencing of the Qa-2 locus (25). Our results indicate that BALB/cJ mice produce a lower number of CD8 α / α TCR- α / β iIELs than the C57BL/6 mice, whereas BALB/cByJ mice produce even fewer TCR- α / β iIELs when compared with C57BL/6 and BALB/cJ mice (Fig. 2). To confirm these results, we examined the iIEL populations present in naturally Qa-2-deficient C3H/HeJ mice, which lack Qa-2 genes altogether (26). We observed that these mice resemble both BALB/cByJ and TAP^{-/-} mice in that only a small number of CD8 α / α TCR- α / β iIELs are present.

To further confirm the apparent importance of Qa-2 in the presence of CD8 α / α TCR- α / β T cells in iIELs, we examined their abundance in Qa-2-transgenic mice. Our data show that Qa-2-transgenic mice produce higher numbers of CD8 α / α TCR- α / β iIELs than do those of their Qa-2⁻ littermates (Fig. 3). Thus, Qa-2 by itself can account for most of the TAP dependence of CD8 α / α TCR- α / β cells among iIELs.

Interestingly, it was found that decreased Qa-2 expression not only led to the reduction of CD8 α / α TCR- α / β cells but also resulted in a reduced number of CD8 α / β TCR- α / β cells (Figs. 2 and 3). Previously, it had been shown that CD8 α / β TCR- α / β cells are dependent on classical MHC class I molecules (17–19). Therefore, the reduction in the number of these cells in Qa-2-deficient mice is certainly not due to their dependence on Qa-2. This reduction could result from (a) CD8 α / β TCR- α / β cells being vulnerable targets of NK cells in the absence of Qa-2 or (b) a dependency of CD8 α / β cells on survival factors that are produced by CD8 α / α TCR- α / β cells. To address the former issue, we examined the F1 animals of a cross between Qa-2⁺ (C57BL/6) and Qa-2⁻ (C3H/He) parents, where the level of Qa-2 expression is intermediate. In these mice, we also found a partial decrease in both the numbers of CD8 α / β TCR- α / β cells and CD8 α / α TCR- α / β cells (Fig. 4). We further confirmed this data by ana-

lyzing an F1 littermate of Qa-2⁺ (C57BL/6) and Qa-2⁻ (BALB/cByJ) parents (not shown). If Qa-2 was important in conferring NK cell inhibitory function, intermediate levels of Qa-2 expression would have been sufficient to protect the CD8 α / β cells, or if the level of Qa-2 was too low to protect from NK cells, then all of the CD8 α / β cells should have been killed. Therefore, this effect is most likely not due to targeting of CD8 α / β TCR- α / β cells by NK cells in the absence of Qa-2. It is of a potential future importance to explore how CD8 α / β cells depend on CD α / α cells for their presence in the gut. It is worth mentioning that an earlier report has shown dependency between two subsets of T cells with regard to their functional maturity (41). Therefore, the presence of CD8 α / β cells in the gut could be dependent on CD8 α / α TCR- α / β cells.

In summary, CD8 α / α TCR- α / β lymphocytes in the intestinal epithelium develop in a manner distinct from that of CD8⁺ T cells in the peripheral lymphoid organs. In particular, in H-2^b mice, CD8 α / α TCR- α / β iIEL selection is independent of the classical MHC class I molecules K^b and D^b. CD1d MHC class Ib products are not involved in this selection process, as shown by the lack of effect of deletion of the CD1d gene. The presence of CD8 α / α TCR- α / β iIELs also requires the expression of TAP- and β _{2m}, indicating that these cells may be selected on TAP- and β _{2m}-dependent class Ib MHC products. This MHC class Ib molecule was shown to be Qa-2, which is the product of up to four MHC class Ib genes in the Q region of the mouse genome. Variation in Qa-2 levels correlated with the level of CD8 α / α TCR- α / β iIELs, and a Qa-2⁻ mouse carrying a Qa-2 transgene had high levels of surface expression of Qa-2 and an excess of CD8 α / α TCR- α / β iIELs.

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