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Expression of CD1d Under the Control of a MHC Class Ia Promoter Skews the Development of NKT Cells, But Not CD8⁺ T Cells

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Although CD1d and MHC class Ia share similar overall structure, they have distinct levels and patterns of surface expression. While the expression of CD1d is known to be essential for the development of NKT cells, the contribution of CD1d to the development of CD8⁺ T cells appears to be inconsequential. To investigate whether CD1d tissue distribution and expression levels confer differential capacity in selecting these two T cell subsets, we analyzed CD8 and NKT cell compartments in Kb-CD1d-transgenic mice that lack endogenous MHC class Ia and CD1d, respectively. We found that MHC class Ia-like expression pattern and tissue distribution are not sufficient for CD1d to rescue the development of CD8⁺ T cells, suggesting that unique structural features of CD1d preclude its active participation in selection of CD8⁺ T cells. Conversely, cell type-specific CD1d surface density is important for the selection of NKT cells, as the NKT cell compartment was only partially rescued by the Kb-CD1d transgene. We have previously demonstrated that increased CD1d expression on dendritic cells enhanced negative selection of NKT cells. In this study, we show that cell type-specific expression levels of CD1d establish a narrow window between positive and negative selection, suggesting that the distinct CD1d expression pattern may be selected evolutionarily to ensure optimal output of NKT cells. The Journal of Immunology, 2003, 171: 4105–4112.

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Abbreviations used in this paper: DC, dendritic cell; WT, wild type; αGalCer, α-galactosylceramide; TEC, thymic epithelial cell; Tg, transgenic; DP, double positive; IF, immunofluorescence.

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as NK1.1 T cells, but not conventional T cell, development is dependent on Fyn (39). The involvement of different cell types and signaling molecules in the positive selection of conventional CD8+ and NK1.1 T cells may in part contribute to the distinct characteristics associated with these two subsets of T cells.

The role of CD1d in the development of other T cell subsets is less well established. No detectable changes were observed in either CD4+ or CD8+ populations in CD1d-deficient mice (40–42), suggesting that the presence of MHC class Ia and class II molecules in these mice may obscure any role of CD1d in selection of conventional T cell populations. Indeed, the number of CD4+ T cells in CD1d-deficient mice is reduced significantly compared with the corresponding population in wild-type mice (43, 44). Although the observed differences between CD1d-deficient and wild-type mice were largely attributed to the loss of CD1d-restricted NK1.1+ T cells, a small population of CD4+ NK1.1+ T cells was also reduced in the CD1d-deficient mice. The majority of these CD1d-restricted CD4+ NK1.1+ T cells express invariant Vα14 TCR. Interestingly, the remaining CD1d-restricted CD4+ NK1.1+ cells also display limited TCR diversity (44). However, in CD1d-deficient mice, as compared with TAP-deficient mice, no significant differences in the CD8+ T cell compartment could be detected (43, 44). Thus, despite the fact that CD8 seems to be a receptor for CD1d (45, 46), CD1d fails to contribute significantly to the development of CD8+ T cells.

Unlike MHC class Ia molecules, which are highly expressed on most cell types, including TECs and DCs, but minimally expressed on immature thymocytes, CD1d is moderately expressed on thymocytes, TECs, and DCs. Thus, it is possible that differential expression patterns of CD1d contribute to its unique role in the selection of NKT cells, while restricting the impact of CD1d in the selection of CD8+ T cells. To characterize the role of ligand density and expression pattern of CD1d in the selection of CD8+ T cells and NKT cells, we have generated CD1d-transgenic mice driven by the H-2Kb promoter in a MHC class Ia-deficient (Kb-Dn) background (Kb, TAP-Tg; H-2Dn) and CD1d background (Kb, TAP-Tg; CD1d-Tg). In this study, we show that no quantitative or qualitative differences in the CD8+ T cell repertoire can be detected between Kb, TAP-Tg Kb, Dn, and Kb-Dn mice, suggesting that CD1d is not sufficient to mediate positive selection of CD8+ T cells, even when expressed at a density and pattern nearly identical to MHC class Ia. Furthermore, the NKT cell compartment is only partially reconstituted in Kb, TAP-Tg Kb, Dn mice, suggesting that altering cell type-specific CD1d expression ablates NKT cell development. Thus, the epitope density threshold defined by the differences in CD1d surface density on positive and negative selection cell types may be extremely important for efficient selection of NKT cells that express a restricted TCR repertoire.

Materials and Methods

Generation of Kb, CD1d-Tg Kb, Dn and Kb, CD1d-Tg Kb, Dn mice

The Kb, CD1d-Tg mouse strains, originally generated in (BALB/c × C57BL/6)F1, were backcrossed six to seven generations onto a C57BL/6 background and described in detail previously (36). The Kb, CD1d-Tg mice were subsequently established on backgrounds deficient for expression of endogenous H-2 class Ia (i.e., H-2Kb and H-2D) or CD1d by crossing transgenic (Tg) mice with either Kb-Dn mice (six generation backcrossed in B6 background) or Cd1d+ mice (12 generations backcrossed in B6 background).

Cell preparation and flow cytometry

The following mAbs were purchased from BD PharMingen (San Diego, CA): FITC-conjugated mAbs specific for CD4 (RM4-5), TCRβ (H57-597), Vβ2 (B20.6), Vβ5 (MR6-4), Vβ6 (RR4-7), Vβ7 (TR310), and Vβ8 (MR5-2); PE-conjugated mAbs specific for CD3ε (145-2C11), B220 (RA3-6B2), CD8α (53-6.7), NK1.1 (PK136), H2-1A9 (MS5114), and CD11c (HL3). Allophycocyanin-CD1d/αGalCer tetramers were prepared as described by Matsuda et al. (47). The CD1d-specific mAb 5C6 (hamster IgG) has been described previously (5). Anti-H-2Kb, Y3, was obtained from the American Type Culture Collection (Manassas, VA). Single-cell suspensions from thymus and spleen were prepared by mechanical desegregation in RPMI 1640 medium containing 10% FBS, 2 mM L-glutamine, 20 mM HEPES, 50 μM 2-ME, nonessential amino acids, sodium pyruvate, penicillin, and streptomycin (RPMI 10). Lymphocytes from perfused liver were obtained according to the method described by Goossens et al. (48). Intraepithelial lymphocytes (IELs) were prepared and purified through discontinuous 40%/70% Percoll gradient centrifugation as described by Tagliabue et al. (49). Thymic stromal cell suspensions were prepared by digesting thymic lobes in 0.1% trypsin and 0.5 mM EDTA for 40 min at 37°C. Digestion was stopped by addition of immunofluorescent (IF) buffer (HBSS containing 2% FBS and 0.1% NaN3). After mechanical disruption of the lobe, cells were harvested and washed twice with IF buffer before cell surface staining experiments. Cells were stained in IF buffer using combinations of fluorescent-conjugated Abs for 30 min at 4°C. When staining involved allophycocyanin-CD1d/αGalCer tetramers, incubation time was extended to 1 h. The stained cells were analyzed by flow cytometry using a FACScan (BD Bioscience, Mountain View, CA) with CellQuest software.

Immunohistochemistry

Thymi were embedded in Tissue-Tek OCT (Miles, Elkhart, IN) and frozen at −80°C. Sections measuring 5–7 μm were cut by using a Leica CM1800 cryostat (Leica, Heerbrugg, Switzerland), air dried at room temperature, and stained. For immunohistochemistry, sections were treated with a saturating concentration of the anti-mouse CD1d (1B1; BD PharMingen), and the Ab bindings were visualized using the ABC Elite system (Vector Laboratories, Burlingame, CA). Slides were counterstained with hematoxylin. Preparations were examined and photographed on an Axioshot 2 apparatus (Zeiss, Thornwood, NY).

Activation of NKT cells and analysis of cytokine production

For examining the functional activity of NKT cells, splenicocytes or hepatic lymphocytes (5 × 105 cells/well) from indicated mice were stimulated either with 100 ng/ml αGalCer or vehicle (0.1% DMSO) in round-bottom microtiter wells in a final volume of 200 μl of RPMI 10. After 48 h, the supernatants were harvested and quantitated by sandwich ELISA. For activation of NKT cell hybridomas, 5 × 105 hybridoma cells were cultured together with irradiated thymocytes or splenocytes (5 × 105 cells/well) in a total volume of 200 μl/well. After 24–48 h, culture supernatants were harvested and IL-2 release was quantitated by ELISA. Abs specific for cytokines and recombinant mouse cytokines were obtained from BD Pharmingen and used according to the manufacturer’s directions.

Adoptive transfer

Bone marrow-derived cells were depleted of T cells by anti-Thy-1.2 (J1j.10; American Type Culture Collection) coupled with rabbit complement (Cedarlane Laboratories, Hornby, Ontario, Canada). Recipient mice received 980 rad for 4 h before injection with donor bone marrow cells. A total of 1 × 107 cells was injected i.v. into recipient mice. Seven weeks after adoptive transfer, splenicocytes and hepatic lymphocytes were collected and the numbers of NKT cells were monitored by flow cytometry as described above.

Statistical analysis

Mean values were compared using Student’s t test for independent variables. Statistical significance was considered to be p < 0.05.

Results

CD1d expression in Kb, CD1d-Tg mice mimics H-2Kb expression

We and others have previously shown that CD1d does not contribute significantly to the development of CD8+ T cells (43, 44). One possible explanation for the limited impact of CD1d on the generation of the CD8+ T cell repertoire is relatively low surface expression and distinct tissue distribution as compared with MHC class Ia molecules. To address this possibility, we generated a Kb, CD1d-Tg transgenic (Tg) mouse, in which the mouse CD1d gene expression is controlled by the classical class I (H-2Kb) promoter (36). Immunohistochemical analysis of thymi of Kb, CD1d-Tg mice
and wild-type (WT) mice showed that K\textsuperscript{b}-CD1dTg thymic medulla expresses much higher levels of CD1d than WT thymus (Fig. 1A). Cortical thymic epithelial cells, which are required for CD8\textsuperscript{+} T cell selection, also express high levels of CD1d in Tg mice (Fig. 1A). Cell surface expression of H-2K\textsuperscript{b} and CD1d in the K\textsuperscript{b}-CD1dTg mice and WT controls was further determined by flow cytometric analysis (Fig. 1B). Compared with WT mice, the expression level of CD1d in K\textsuperscript{b}-CD1dTg mice is much higher in most of the cell types examined, including mature (CD3\textsuperscript{high}) thymocytes, splenic B and T cells, and thymic and intestinal epithelial cells. The levels CD1d expressed on CD3\textsuperscript{low} Tg cortical thymocytes, however, is comparable to the WT mice, as the contribution of the K\textsuperscript{b}-CD1d transgene is minimal on immature thymocytes (Fig. 1B). Overall, the expression pattern of CD1d in the K\textsuperscript{b}-CD1dTg mice is largely contributed by the K\textsuperscript{b}-CD1d transgene, which mimics the expression pattern of H-2K\textsuperscript{b} molecules.

The development of CD8\textsuperscript{+} T cells cannot be rescued in K\textsuperscript{b}-CD1dTgK\textsuperscript{b0}D\textsuperscript{b0} mice

The K\textsuperscript{b}-CD1dTg mice were crossed with K\textsuperscript{b}D\textsuperscript{b}-deficient mice (K\textsuperscript{b0}D\textsuperscript{b0}) and the effect of the CD1d transgene on the development of the CD8 compartment was examined. The absence of H-2 class Ia expression eliminates possible competition with the CD1d transgene that could limit CD1d-dependent thymic selection of CD8\textsuperscript{+} T cells.
T cells. We examined the size of the CD4+ and CD8+ T cells pools in Kb-CD1dTgKboDbo and KboDbo mice by flow cytometric analysis. Compared with the WT control, the CD8+ population was significantly depleted in KboDbo mice. This CD8+ population was not reconstituted in Kb-CD1dTgKboDbo mice, as the number of residual CD8+ T cells in KboDbo mice is similar to that in Kb-CD1dTgKboDbo in all organs tested, including thymus, spleen, liver, and lymph nodes (Fig. 2 and Table I). The NK cell population was decreased in Kb-CD1dTgKboDbo mice, presumably due to the enhanced negative selection, as previously reported (36). A decrease in the CD4 compartment in the liver of the Kb-CD1dTgKboDbo mice was also observed. This decrease is likely due to the loss of CD4+ CD1d-restricted NKT cells. In addition, no significant difference was found in the CD8aαTCRαβ+ and CD8αβTCRβ+ IEL subpopulation between Kb-CD1dTgKboDbo and KboDbo mice (data not shown).

To determine whether residual T cells selected in Kb-CD1dTgKboDbo mice are phenotypically different from those in KboDbo mice, we compared the TCR Vβ usage of Kb-CD1dTgKboDbo mice with the peripheral CD8+ T cells in Kb-CD1dTgKboDbo and KboDbo mice. Fig. 3 shows that the splenic CD8+ T cells in Kb-CD1dTgKboDbo mice display a similar spectrum of Vβ usage to that of KboDbo mice. Thus, it is unlikely that the residual CD8+ T cells in Kb-CD1dTgKboDbo mice are of a different origin than those in KboDbo mice. In addition, the percentage of CD8+ T cells that express activated/memory phenotype (e.g., CD44 high) is comparable between Kb-CD1dTgKboDbo and KboDbo mice (data not shown). Taken together, our data suggest that CD1d does not contribute significantly to the positive selection of conventional CD8+ T cells, even at comparable expression levels and pattern with MHC class Ia.

Partial reconstitution of NKT cells in Kb-CD1dTgCD1+ mice

We have previously demonstrated that the high ligand density of CD1d on hemopoietic-derived cells (e.g., DCs), but not on non-hemopoietic cells, can mediate negative selection of NKT cells (36). As a consequence of enhanced negative selection, Kb-CD1dTg mice have a greatly reduced NK cell compartment as compared with WT mice. To determine whether expression of CD1d under the control of the H-2Kb promoter can rescue the positive selection of NKT cells, we crossed the KboCD1dTg mice onto the CD1d-deficient (CD1−) background. Since the Kb-CD1d transgene is expressed on most cell types tested, including double-positive (DP) thymocytes, one would expect that the expression of the Kb-CD1d transgene in CD1− mice could lead to positive selection of NKT cells. Indeed, a small but highly reproducible population of CD1dαGalCer tetramer-positive NKT cells was observed in Kb-CD1dTgCD1+ mice but in not CD1+ mice (Fig. A4). However, this population by no means completely reconstitutes the NKT cell compartment. Furthermore, the reconstituted population in Kb-CD1dTgCD1+ mice is significantly smaller than the residual population in Kb-CD1dTgCD1− mice (Fig. A4B). Thus, the incomplete reconstitution in Kb-CD1dTgCD1+ mice cannot be explained solely by negative selection of the NKT cells. Endogenous CD1d must play some role in the efficient positive selection of NKT cells.

To examine whether CD1d expressed in Kb-CD1dTgCD1+ mice can present Ag efficiently and be recognized by NKT cells, thymocytes and splenocytes isolated from Kb-CD1dTgCD1+ and WT mice were compared for their ability to present αGalCer to CD1d-restricted NKT cell hybridomas. As shown in Fig. 5, NKT cell hybridomas secreted high levels of IL-2 in response to αGalCer presented either by cells isolated from Kb-CD1dTgCD1+ mice or WT mice. Thus, there is no qualitative difference between CD1d expressed on Kb-CD1dTgCD1+ mice and WT mice with regard to Ag presentation and recognition by NKT cells.

To investigate whether the Kb-CD1d transgene expressed on hemopoietic cells can support the positive selection of CD1d-restricted NKT cells, we established bone marrow chimeras among KboCD1dTgCD1−, WT, and CD1+ mice. The Tg“CD1+”→CD1− chimeras yielded NKT cell populations similar in frequency to those of Tg“CD1−”→WT chimeras, demonstrating that, similar to endogenous CD1d, the Kbo-driven expression of CD1d transgene on hemopoietic cells can contribute to positive selection of NKT cells, and the expression of endogenous CD1d on nonhemopoietic cells did not enhance the selection/development of NKT cells. However, the Tg“CD1+”→WT bone marrow chimeras had significantly lower numbers of NKT cells and significantly reduced capacity to secrete IFN-γ and IL-4 upon stimulation with αGalCer than did WT→WT or WT→Tg“CD1+” chimeras (Fig. 6), possibly due to the excessive negative selection of NKT cells mediated by higher

### Table 1. Frequency of lymphocyte subpopulations in KboDbo and Kb-CD1dTgKboDbo mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Phenotype</th>
<th>KboDbo (%)</th>
<th>Kb-CD1dTgKboDbo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymus</td>
<td>CD8+</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>CD4+</td>
<td>6.8 ± 1.9</td>
<td>6.4 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>NKT</td>
<td>1.2 ± 0.2</td>
<td>0.3 ± 0.1*</td>
</tr>
<tr>
<td>Spleen</td>
<td>CD8+</td>
<td>2.1 ± 0.4</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>CD4+</td>
<td>21.7 ± 3.0</td>
<td>24.6 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>NKT</td>
<td>1.7 ± 0.9</td>
<td>0.28 ± 0.1*</td>
</tr>
<tr>
<td>Liver</td>
<td>CD8+</td>
<td>3.1 ± 0.6</td>
<td>3.67 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>CD4+</td>
<td>26.5 ± 2.4</td>
<td>19.6 ± 3.7*</td>
</tr>
<tr>
<td></td>
<td>NKT</td>
<td>17.3 ± 4.6</td>
<td>8.6 ± 1.7*</td>
</tr>
</tbody>
</table>

*Values represent mean ± SD. Results obtained from six mice per group. No significant difference in total cell number of various organs is detected between KboDbo and Tg“KboDbo” mice.
CD1d expression on some Tg hemopoietic cells (i.e., DCs) as described previously in K^b-CD1dTgCD1^wt chimeras (36). Thus, redirecting CD1d expression under the control of class Ia does not appear to affect the cell type requirements for the selection of NKT cells.

One possible explanation for the limited reconstitution of NKT cells in K^b-CD1dTgCD1^o mice would be a cell type-specific threshold of CD1d expression for positive and negative selection. As mentioned earlier, although H-2K^b is highly expressed in many cell types, cortical thymocytes express relatively low levels of H-2K^b. In fact, in K^b-CD1dTgCD1^o mice, the expression of Tg CD1d on cortical thymocytes necessary for positive selection is merely comparable to the expression level of endogenous CD1d (Fig. 7). In the case of K^b-CD1dTgCD1^wt mice, the expression of CD1d on K^b-CD1dTgCD1^wt thymocytes is significantly higher (3-fold) than endogenous CD1d (Fig. 7). Thymic DCs, which mediate negative selection, however, express 20- to 40-fold higher CD1d than WT thymic DCs in both K^b-CD1dTgCD1^wt and K^b-CD1dTgCD1^o mice (Fig. 7). These results suggest that the ultimate size of the NKT cell compartment may be determined by the relative ratio of CD1d expressed on cell types that mediate positive and negative selection of NKT cells. The incomplete reconstitution of the NKT cells compartment in K^b-CD1dTgCD1^o mice is likely a consequence of the alteration of this ratio due to the K^b-like expression pattern of CD1d. Taken together, our data suggest that the distinct expression pattern of CD1d on the hemopoietic cells is important for optimal outcome of the NKT cell selection.

Discussion

Previous studies have shown that MHC class Ia and CD1d play a nonredundant role in the development of CD8 and NKT cells. However, it was not clear whether unique structural features and/or distinct tissue distribution of CD1d contribute to its differential capacity in the selection of CD8 and NKT cells. To address the issue, we have analyzed CD8 and NKT cell compartments in H-2K^b promoter-driven CD1d Tg mice. We have found that CD1d is not sufficient even when mimicking the K^b expression pattern to mediate positive selection of CD8^+ T cells. This finding suggests that the intrinsic characteristic(s) rather than insufficient expression of endogenous CD1d may limit the capacity of CD1d in the selection of CD8^+ T cells in the selection of conventional CD8^+ T cells in WT mice. The distinct pattern and levels of CD1d expression, however, are critical for the selection of NKT cells. Altering CD1d expression to mimic MHC class Ia leads to inefficient NKT cell development. Thus, tight regulation of CD1d expression is particularly important for NKT cell development.

Our finding that the expression of a K^b-CD1d transgene fails to lead to development of a significant CD8^+ T cell population in class Ia-deficient mice is somewhat surprising, given that the overall structure of CD1d closely resembles MHC class Ia. Several recent studies have demonstrated the involvement of MHC class Ib molecules, such as H-2M3 and Qa-1, in the positive selection of CD8^+ T cells (50–52). However, it is noteworthy that the sequence similarity between CD1d and MHC class Ia is only 30–35%, while other CD8 T cell-selecting MHC class I b molecules share 60–70% sequence similarity with MHC class Ia. Several possible explanations could account for the failure of K^b-driven CD1d in participating in the selection/maintenance of the CD8^+ T cell repertoire. First, CD1d may interact less effectively with CD8 than other class I molecules and, as a result, fail to select a significant number of developing T cells to the CD8 lineage. Mouse CD1d has been demonstrated to bind CD8 in redirected CTL assays (45); however, the affinity of CD1d for CD8 appears to be...
hybridomas (DN3A4 and D32.D3) were cultured with 5 × 10^5 endogenous H2-Kb levels, which do not result in excessive negative selection of thymic DCs in these mice are similar to the endogenous CD1d expression levels in B6 mice. We have also previously shown that expression of CD1d in NKT cell development. We predicted that NKT cells could be positively selected as efficiently pos-
itive selection of NKT cells. Because of the restricted TCR repertoire of NKT cells, the cell type-specific expression levels of CD1d in K^b^-CD1dTgCD1^o mice may be required for efficient positive selection. Either positive selection is enhanced in K^b^-CD1dTgCD1^o mice due to greater levels of CD1d on thymocytes (Fig. 7) and/or positive selection is less effective in K^b^-CD1dTgCD1^o mice due to the differences in the pattern and timing of expression between endogenous and K^b^-driven CD1d. During embryonic development, the levels of K^b^ expression decreases progressively on immature thymocytes between gestational day 16 and 1 wk old, and the low levels of K^b^ continue to be expressed on cortical thymocytes through adulthood (5). In contrast, no difference in the level of endogenous CD1d expression on thymocytes could be detected from gestational day 16 onward (5, 54). Since a significant number of CD1d-restricted NKT cells are not detected in thymus until 5 days after birth (55), the expression of endogenous CD1d may be crucial during early development for efficient NKT cell selection. Thus, it is possible there is a time window during the course of development for high efficiency positive selection of NKT cells.

Interestingly, however, the reconstituted NKT cell population in K^b^-CD1dTgCD1^o is significantly and reproducibly smaller than the residual NKT cells in K^b^-CD1dTgCD1^o mice (Fig. 4B). It is unlikely that the K^b^-driven CD1d is functionally different from endogenous CD1d since lymphocytes from K^b^-CD1dTgCD1^o mice can be recognized by and activate NKT cell hybridomas. In addition, hemopoietic cells from K^b^-CD1dTgCD1^o are capable of mediating positive selection of NKT cells in bone marrow chimeras. One explanation for the larger NKT cell population in K^b^-CD1dTgCD1^o is that the presence of endogenous CD1d must be required for efficient positive selection. Either positive selection is enhanced in K^b^-CD1dTgCD1^o mice due to greater levels of CD1d on thymocytes (Fig. 7) and/or positive selection is less effective in K^b^-CD1dTgCD1^o mice due to the differences in the pattern and timing of expression between endogenous and K^b^-driven CD1d. During embryonic development, the levels of K^b^ expression decreases progressively on immature thymocytes between gestational day 16 and 1 wk old, and the low levels of K^b^ continue to be expressed on cortical thymocytes through adulthood (5). In contrast, no difference in the level of endogenous CD1d expression on thymocytes could be detected from gestational day 16 onward (5, 54). Since a significant number of CD1d-restricted NKT cells are not detected in thymus until 5 days after birth (55), the expression of endogenous CD1d may be crucial during early development for efficient NKT cell selection. Thus, it is possible there is a time window during the course of development for high efficiency positive selection of NKT cells.

Our data indicate that ligand density and cell type-specific expression of CD1d is important for NKT cell selection. A very tight threshold exists between the presence and absence of endogenous CD1d since lymphocytes from K^b^-CD1dTgCD1^o mice are capable of mediating positive selection of NKT cells in bone marrow chimeras. One explanation for the larger NKT cell population in K^b^-CD1dTgCD1^o is that the presence of endogenous CD1d must be required for efficient positive selection. Either positive selection is enhanced in K^b^-CD1dTgCD1^o mice due to greater levels of CD1d on thymocytes (Fig. 7) and/or positive selection is less effective in K^b^-CD1dTgCD1^o mice due to the differences in the pattern and timing of expression between endogenous and K^b^-driven CD1d. During embryonic development, the levels of K^b^ expression decreases progressively on immature thymocytes between gestational day 16 and 1 wk old, and the low levels of K^b^ continue to be expressed on cortical thymocytes through adulthood (5). In contrast, no difference in the level of endogenous CD1d expression on thymocytes could be detected from gestational day 16 onward (5, 54). Since a significant number of CD1d-restricted NKT cells are not detected in thymus until 5 days after birth (55), the expression of endogenous CD1d may be crucial during early development for efficient NKT cell selection. Thus, it is possible there is a time window during the course of development for high efficiency positive selection of NKT cells.

FIGURE 5. Recognition of K^b^-CD1dTgCD1^o thymocytes by NKT cell-derived hybridomas. Briefly, 5 × 10^5 cells of CD1d-specific NKT cell hybridomas (DN3A4 and D32.D3) were cultured with 5 × 10^5 thymocytes from K^b^-CD1dTgCD1^o (Tg^CD1^o) mice or WT mice in the presence or absence of αGalCer. IL-2 levels in the supernatant were detected by ELISA. NKT cell hybridomas cultured with thymocytes from CD1^o mice did not secrete detectable amounts of IL-2 (<1 U/ml). Similar results were obtained when splenocytes from indicated mice were used as stimulators (data not shown). Bars represent means and SDs of duplicate determinations. Results are representative of two experiments.

FIGURE 6. K^b^-CD1d expressed on bone marrow-derived cells determines the selection outcome of NKT cells. Bone marrow-derived cells (1 × 10^5) from WT, Tg^CD1^o, and CD1^o mice were transferred into lethally irradiated recipient mice (980 rad) by i.v. injection. After 7 wk, hepatic lymphocytes from bone marrow chimeras were isolated, stained with FITC- anti-TCR and PE-NK1.1, and analyzed by flow cytometry. Absolute NKT cell numbers of hepatic lymphocytes from each group of bone marrow chimeras were calculated as described above. Cytokine production by hepatic lymphocytes from each group of bone marrow chimeras was determined by culturing 5 × 10^5 hepatic lymphocytes from the indicated group of mice with 100 ng/ml αGalCer for 48 h. IFN-γ and IL-4 levels in the supernatant were detected by ELISA. Data shown represent mean ± SE of three mice in each group.

Interestingly, however, the reconstituted NKT cell population in K^b^-CD1dTgCD1^o is significantly and reproducibly smaller than the residual NKT cells in K^b^-CD1dTgCD1^o mice (Fig. 4B). It is unlikely that the K^b^-driven CD1d is functionally different from endogenous CD1d since lymphocytes from K^b^-CD1dTgCD1^o mice can be recognized by and activate NKT cell hybridomas. In addition, hemopoietic cells from K^b^-CD1dTgCD1^o are capable of mediating positive selection of NKT cells in bone marrow chimeras. One explanation for the larger NKT cell population in K^b^-CD1dTgCD1^o is that the presence of endogenous CD1d must be required for efficient positive selection. Either positive selection is enhanced in K^b^-CD1dTgCD1^o mice due to greater levels of CD1d on thymocytes (Fig. 7) and/or positive selection is less effective in K^b^-CD1dTgCD1^o mice due to the differences in the pattern and timing of expression between endogenous and K^b^-driven CD1d. During embryonic development, the levels of K^b^ expression decreases progressively on immature thymocytes between gestational day 16 and 1 wk old, and the low levels of K^b^ continue to be expressed on cortical thymocytes through adulthood (5). In contrast, no difference in the level of endogenous CD1d expression on thymocytes could be detected from gestational day 16 onward (5, 54). Since a significant number of CD1d-restricted NKT cells are not detected in thymus until 5 days after birth (55), the expression of endogenous CD1d may be crucial during early development for efficient NKT cell selection. Thus, it is possible there is a time window during the course of development for high efficiency positive selection of NKT cells.

Our data indicate that ligand density and cell type-specific expression of CD1d is important for NKT cell selection. A very tight threshold exists between the presence and absence of endogenous CD1d since lymphocytes from K^b^-CD1dTgCD1^o mice are capable of mediating positive selection of NKT cells in bone marrow chimeras. One explanation for the larger NKT cell population in K^b^-CD1dTgCD1^o is that the presence of endogenous CD1d must be required for efficient positive selection. Either positive selection is enhanced in K^b^-CD1dTgCD1^o mice due to greater levels of CD1d on thymocytes (Fig. 7) and/or positive selection is less effective in K^b^-CD1dTgCD1^o mice due to the differences in the pattern and timing of expression between endogenous and K^b^-driven CD1d. During embryonic development, the levels of K^b^ expression decreases progressively on immature thymocytes between gestational day 16 and 1 wk old, and the low levels of K^b^ continue to be expressed on cortical thymocytes through adulthood (5). In contrast, no difference in the level of endogenous CD1d expression on thymocytes could be detected from gestational day 16 onward (5, 54). Since a significant number of CD1d-restricted NKT cells are not detected in thymus until 5 days after birth (55), the expression of endogenous CD1d may be crucial during early development for efficient NKT cell selection. Thus, it is possible there is a time window during the course of development for high efficiency positive selection of NKT cells.

Our data indicate that ligand density and cell type-specific expression of CD1d is important for NKT cell selection. A very tight regulation of NKT cell development is not surprising considering the broad role of NKT cells in controlling infection and autoimmunity as well as their rapid activation to secrete large amounts of IFN-γ and/or IL-4 (56). In our K^b^-CD1d Tg system, even the difference between the presence and absence of endogenous CD1d results in differential selection of NKT cells. Because of the restricted TCR repertoire of NKT cells, the cell type-specific expression levels of CD1d may establish a narrow threshold for NKT cell selection. Thus, it is possible there is a time window during the course of development for high efficiency positive selection of NKT cells.

Evolutionarily, the necessity of cell type-specific or time-specific expression of CD1d in NKT selection is supported by the
The distinct cell type-specific expression levels of CD1d, as well as maintenance and genotyping the mice.

Zimmer for critical reading for this manuscript, and T. King for help in obtaining the results in loss of the majority of the NKT cell population suggests a unique subset of major histocompatibility complex class II-dependent helper cells in the generation of CD8 T cells.

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The CD1d-independent NK T cells.

tumor surveillance by natural killer (NK) and NKT cells. Science 278:1623.

The cluster of differentiation of CD1d. Despite structural and sequence similarity to MHC class Ia, class Ib, and even class II molecules, the region flanking the 5’ transcriptional start site of CD1d shares little homology with these other molecules (Y. Geng and C.-R. Wang., unpublished results). Additionally, CD1d genes are located outside of the MHC in both mice and humans (3, 57). The segregation of these two gene complexes during evolution may facilitate the development of different regulatory mechanisms to control the expression of these Ag-presenting molecules. Our finding that changing the CD1d expression pattern in our K b -CD1dTg mice results in loss of the majority of the NKT cell population suggests that there may be selective pressures for the different cell-specific regulation of CD1d separate from other Ag-presenting molecules. The distinct cell type-specific expression levels of CD1d, as well as structural divergence of CD1d from other MHC class I-like molecules, underlines the nonredundant role of CD1d and MHC molecules in the development of the T cell repertoire.

Acknowledgments

References


