Development of Intestinal Intraepithelial Lymphocytes, NK Cells, and NK 1.1\(^+\) T Cells in CD45-Deficient Mice

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The transmembrane protein tyrosine phosphatase CD45 is differentially required for the development and function of B, T, and NK cells, with mice partially deficient for CD45 having a significant inhibition of T cell, but not NK or B cell, development. CD45-mediated signaling has also been implicated in the development of intrathymic, but not extrathymic, intestinal intraepithelial T lymphocytes (iIELs) in the CD45ex6−/− mouse. As NK1.1+ CD3+ (NK-T) cells can also develop through extrathymic pathways, we have investigated the role of CD45 in NK-T cell development. In mice with a complete absence of CD45 expression (CD45ex9−/−), the NK-T cell population was maintained in the iIEL compartment, but not in the spleen. Functionally, CD45-deficient NK-T cells were unable to secrete IL-4 in response to TCR-mediated signals, a phenotype similar to that of CD45-deficient iIELs, in which in vitro cytokine production was dramatically reduced. Using the CD45ex9−/− mouse strain, we have also demonstrated that only one distinct population of NK-T cells (CD8+) appears to develop normally in the absence of CD45. Interestingly, although an increase in cytotoxic NK cells is seen in the absence of CD45, these NK cells are functionally unable to secrete IFN-γ. In the absence of CD45, a significant population of extrathymically derived CD8αα+ iIELs is also maintained. These results demonstrate that in contrast to conventional T cells, CD45 is not required during the development of CD8αα+ NK-T cells, NK cells, or CD8αα+ iIELs, but is essential for TCR-mediated function and cytokine production. The Journal of Immunology, 2001, 166: 6066–6073.

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onventional T cell subsets depend upon the thymus for their development; however, other lineages, such as CD8αα+ intestinal intraepithelial lymphocytes (iIELs), CD8αβ+ NK1.1+ CD3+ T cells, and NK cells develop normally in athymic (nu) mice (1–7). The development of these lineages appears to be controlled by IFN regulatory factor 1 and its regulation of IL-15 gene expression (8–12). The iIEL compartment contains a rich and phenotypically diverse T lymphocyte population whose size, by some estimates, rivals that of the pool of T lymphocytes in the spleen (13). All iIEL express the ααβ, ααβ integrin that binds E-cadherin and mediates adherence to epithelial cells (14–16). The vast majority of the iIEL T lymphocyte population expresses the CD8α-chain; however, only ~20% of these lymphocytes express the CD8β-chain (17). CD8αβ heterodimer expression is characteristic of thymus-derived CD8αα T lymphocytes, while CD8αα homodimer expression has been associated with extrathymically derived T lymphocytes in nude mice and in thymectomy models (18–20). Both TCRαβ and TCRγδ T lymphocytes in the iIEL can express the CD8αα homodimer, whereas only TCRαβ+ T lymphocytes express the CD8αβ heterodimer (13). Thus, TCRγδ+ and a portion of the TCRαβ+ iIEL population are considered to be of extrathymic origin. The site of extrathymic iIEL maturation has not been clearly established, although there is evidence that the intestine is involved (21–25). Functionally, iIEL are known to make Th1 cytokines (IL-2 and IFN-γ) (26).

NK cells represent a population of lymphocytes that can lyse tumor cells that may lack MHC class I expression. NK cells share multiple surface molecules with T cells and may be generated from common precursors; however, functional NK cells can develop in the absence of a thymus (27). Additionally, there is a subset of lymphocytes that share receptor structures common to both T cell and NK cell lineages, the NK1.1+ CD3+ CD8αα cell (NK-T cell) (3, 5, 28). This lymphocyte population coexpresses the αβ TCR and the NK surface receptor NKR-P1 (NK1.1 in the C57BL/6 mouse strain). There are two phenotypically and functionally distinct subsets of NK-T cells described in most tissues. One population is CD44+ or CD44−CD8− (double negative) and is CD1d and thymus dependent, while the CD8+ population is CD1d and thymus independent. Most CD1d- and thymus-dependent NK-T cells use one of only three TCR Vβ domains (Vβ8.2, Vβ2, or Vβ2) and a single Vα domain (Vα14) (5, 28, 29). In addition to this restricted TCR repertoire, CD1d- and thymus-dependent NK-T cells have the other unusual property of rapidly releasing IL-4 within 1 h of TCR engagement. In contrast, CD8− NK-T cells express heterogeneous TCR, do not produce IL-4 rapidly (7), and can develop extrathympically from fetal liver precursors (3, 7).

The transmembrane protein tyrosine phosphatase CD45 (leukocyte common Ag, Ly-5) has been shown to be critically important for thymic T lymphocyte development (30, 31). CD45 is expressed on all nucleated cells of hematopoietic origin, including B, T, and NK cells (32). Multiple isoforms of CD45 exist, ranging in size...
CD45 exon 9 deficient, CD45ex9, which exon 12 was replaced, and another in which exon 9 was replaced. In spleen and lymph nodes. The CD45ex9, which this low level CD45 expression had on CD8+ T cell function, groups of two mice were given 2 μg of anti-CD3 (2C11) by i.v. injection. The spleens were removed after 90 min, and 1 × 10^7 splenocytes/ml were cultured in R10 for 2 h. For the analysis of iIEL NK-T cell function, iIELs were isolated as described above, and 1 × 10^5 cells were cultured for 4 h at 37°C in 5% CO₂ in the presence of 100 μg/ml anti-CD3 (2C11) in R10. IL-4 levels were measured in culture supernatants using a mouse IL-4 ELISA kit (PharMingen). IL-2-activated NK cell (LAK) preparations were performed as previously described (40). Briefly, splenocyte suspensions were made in HBSS and 10% FCS. After RBC lysis, washed cells were incubated in a nylon-wool column for 45 min at 37°C. Nonadherent cells were cultured in RPMI 1640 with 10% FCS, Glutamax I, penicillin-streptomycin, 2-ME, and 100 U/ml of rIL-2 (Chiron, Emeryville, CA) at 37°C. On day 3 adherent cells were removed with Versene (Life Technologies) and washed. The cells were then incubated in 1/1 dilutions of culture supernatants for 18 h (anti-CD4) and YTS169.4 (anti-CD8) Abs and a 1/15 dilution of Low Tox M Rabbit Complement (Cederlane Laboratories, Hornby, Ontario, Canada) for 45 min at 37°C. After washing, the remaining cells were expanded for 2–4 days in 800 U/ml of rIL-2 at 4 × 10^6 cells/ml.

Two assays of NK cell activity were performed. Natural killing assays were performed as previously described (40). Briefly, tumor targets were radiolabeled with 32P (25 μCi/10^6 cells) for 90 min in the absence of FCS. Effector cells were added to 96-well round-bottom plates at densities sufficient to achieve the indicated E:T cell ratios. Radiolabeled targets were added (105/well) and incubated for 4 h at 37°C. One hundred and fifty microliters of supernatant was harvested and assayed for 32P release. Specific cysis was determined as follows: % specific lysis = 100 × (exp spont)/(max spont – spont).

In vitro IFN-γ release by NK cells was performed as previously described (41). Briefly, 96-well flat-bottom plates were coated with goat anti-
mouse F(ab')2 (Jackson ImmunoResearch) and then mouse anti-NK1.1 at the indicated concentrations. NK cell populations were incubated for 4 h at 37°C at 2 × 10^6 cells/well. IFN-γ was measured in culture supernatants using a mouse IFN-γ ELISA kit (PharmaMingen).

Results

NK cells are increased, while NK-T cells are decreased, in spleens of CD45−/− mice

Through the use of flow cytometry, we examined whether NK and NK-T cells could develop in CD45-deficient mice. An increase in NK cells was previously described in CD45ex6−/− mice; however, as these mice express low levels of CD45, we wanted to evaluate the numbers of NK cells in the completely CD45-deficient CD45ex9−/− mouse. As shown in Table I, there was a 2-fold increase in the percentage of NK cells in the spleens of CD45ex9−/− mice. This translates into a significant (3.5-fold) increase in the total number of splenic NK cells. This substantial change in NK cells is in contrast to the dramatic decrease in NK-T cell percentages and numbers in the spleens of CD45-deficient mice. As shown in Table I, CD3+ NK1.1+ cells were at the limit of reliable detection in CD45ex9−/− mice (>10-fold decreased). This decrease precluded the identification of NK-T cell subpopulations in the spleens of CD45ex9−/− mice.

Intestinal IEL are present in CD45ex9−/− mice

Enriched iIELs were stained for αIEL expression after harvest, and αIEL-positive cells were detected by flow cytometry. In C57BL/6 mice, 12.3 ± 1.5% of the total intestinal preparation (iIEL and enterocytes) was positive for αIEL, whereas in CD45ex6−/− and CD45ex9−/− mice only 3.9 ± 0.8 and 2.6 ± 0.6%, respectively, of the intestinal preparation were αIEL-positive. Based on these percentages and the total number of cells (epithelial and iIEL) in the preparations, the absolute number of iIELs in the respective populations was calculated (Table II). A decrease in the absolute number of iIELs was observed in both CD45ex6−/− and CD45ex9−/− mice (Table II and Fig. 1A).

Phenotype of iIEL in CD45ex6−/− mice

Because of the presence of a significant, albeit reduced, population of iIEL in the CD45ex6−/− and CD45ex9−/− mouse strains, further phenotypic characterization was performed. To normalize for number of iIEL, lymphocytes were stained for αIEL and the marker of interest. Thus, all values are the percentage iIEL (αIEL-positive) cells positive for the second marker, allowing for comparison between C57BL/6 and deficient mice.

All iIEL from C57BL/6 mice expressed CD45, while only 25% of iIEL from the CD45ex6−/− mice express CD45 (Fig. 1, B–D). This percentage is higher than the low, but detectable, levels of expression in other lymphoid compartments of CD45ex6−/− mice (CD45ex6−/− splenocytes, 3–4% CD45). It should also be noted that the intensity of CD45 expression on iIELs was slightly decreased (mean fluorescence intensity: C57BL/6, 1299; CD45ex6−/−, 568). iIEL from the CD45ex9−/− strain did not express CD45 (Fig. 1, B and E), thus confirming the complete absence of CD45 protein expression in this mutant strain.

With respect to CD8 expression, 79% of the iIEL in the CD45ex6−/− mice and 77.5% of the iIEL in the CD45ex9−/− mice expressed the CD8α chain compared with 86% in the C57BL/6 control (Table III and Fig. 2A). However, while 12.9% of the CD45ex6−/− iIEL and 15% of the C57BL/6 iIEL expressed the CD8β chain, only 4.5% of iIELs in an age-matched CD45ex9−/− mouse were CD8β+ (Fig. 2B and Table III). To determine whether there was attenuation of TCRαβ or TCRγδ populations, iIEL were costained for αIEL and either TCRαβ or TCRγδ. Both T lymphocyte populations were present in the CD45ex6−/− and CD45ex9−/− animals, although with slightly decreased percentages compared with C57BL/6 (Table III). The majority of CD45-positive cells in the CD45ex6−/− iIELs (94%) are TCRαβ positive (Fig. 3A). As previously described, there was an increase in CD4-positive iIELs in the CD45ex6−/− mouse; however, this elevation was not seen in the CD45ex9−/− iIELs, indicating a decrease in CD4-positive iIELs in the complete absence of CD45.

The percentage of NK and NK-T cells is increased in iIELs in CD45-deficient mice

To examine whether part of the retained iIELs in CD45-deficient mice were NK and/or NK-T cells, we analyzed the dual expression of NK1.1 and CD3 on iIELs by flow cytometry. As shown in Fig. 4A, C57BL/6 mice have a minor population (mean ± SE, 7.8 ± 0.8) of NK1.1+CD3+ cells in the iIEL population. This population of NK-T cells is significantly elevated in the CD45ex6−/− (12.5 ± 4.6%) and CD45ex9−/− (12.3 ± 1.4%) iIEL populations (Table I and Fig. 4). In addition, although the normal iIEL population has almost no measurable NK cells (1.8 ± 0.6%), the iIEL populations from CD45-deficient mice have a significantly increased percentage of NK cells (Table I and Fig. 4; CD45ex6−/−, 5.9 ± 2.4%; CD45ex9−/−, 8.8 ± 2.5%). This increase in the percentage of NK cells in the iIEL population results in an increase in the total number of NK cells in the iIEL population that is similar to the fold increase in the total number of NK cells in the spleen.

As two clearly distinct populations of NK-T cells have been described, we used flow cytometric analysis to analyze whether there was selective survival of these subpopulations in CD45ex9−/− iIELs. In the C57BL/6 spleen, the majority of NK-T

<table>
<thead>
<tr>
<th>Table I.</th>
<th>The development of NK and NK-T cells in the spleen and iIEL of CD45ex9-deficient mice</th>
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<tbody>
<tr>
<td>Mice</td>
<td>Spleen</td>
</tr>
<tr>
<td></td>
<td>CD45ex9−/−</td>
</tr>
<tr>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>81 ± 3</td>
</tr>
<tr>
<td>% NK1.1+</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>CD3</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>% NK1.1+</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>CD3</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>% NK1.1+</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>CD4</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

a Total spleen cells and iIELs were isolated, counted, and stained with hamster anti-CD3 and biotinylated NK1.1 mAb, followed by FITC anti-hamster and streptavidin-PE or biotinylated NK1.1 mAb, followed by streptavidin-PE and FITC anti-CD4 or CD8a. Percentages of cells in whole spleen were analyzed by flow cytometry, and numbers of each population were calculated (mean ± SEM). Results are means of four separate experiments (n = 6, C57BL/6; n = 3, CD45ex9−/−). *p < 0.05 for C57BL/6 vs CD45ex9−/− mice.

b BDL, Below detection limit.
cells were CD4+ or CD4−CD8−, in agreement with previous reports (Table I; 7, 42). Surprisingly, however, as is shown in Table I and Fig. 4, almost all NK-T cells in the iIEL compartment were CD8+. When these results were compared with the subpopulations of NK-T cells in the CD45ex6−/− iIEL compartments, there was a retention of the CD8+ NK-T cell population (Table I). There was no clearly defined population of CD4+ or CD4−CD8− NK-T cells in the iIEL compartment (Fig. 4).

**Functional activity of CD45-deficient iIELs, NK-T cells, and NK cells**

To test the functional capability of the remaining iIEL in the CD45ex6−/− and CD45ex9−/− mice, the production of IL-2 in response to immobilized anti-TCR complex Abs was performed. Proliferation of the IL-2-dependent CTLL cell line was measured. Although incubation of C57BL/6 iIEL with anti-CD3, anti-TCRαβ and anti-TCRγδ Abs resulted in significant IL-2 production (Fig. 3B and data not shown), minimal IL-2 production was activated by Ab-mediated TCR stimulation in CD45ex6−/− or CD45ex9−/− iIEL (Fig. 3B). Therefore, the reduced number of iIELs in CD45-deficient strains is coupled with a significant impairment of iIEL function measured in vitro.

As shown above, the total number of splenic T cells bearing the NK-T cell phenotype by FACS analysis was decreased >10-fold in CD45-deficient mice. To determine whether there were cells capable of NK-T cell function in the absence of cells expressing the typical NK-T cell phenotype profile, we assessed IL-4 production after i.v. administration of anti-CD3 mAb (Fig. 5A). A complete loss of NK-T cell function was demonstrated in both the CD45ex6−/− and CD45ex9−/− strains. When iIEL NK-T cell function was analyzed in a similar fashion, low amounts of IL-4 production could be measured in the C57BL/6 population, while no cytokine production could be measured in the CD45ex6−/− population (Fig. 5B). Therefore, although these mouse strains demonstrate that iIEL NK-T cells can develop in the absence of CD45 cell surface expression, these NK-T cells are unable to perform the function of rapid IL-4 release after anti-CD3 stimulation.

We also used the CD45ex9−/− mouse strain to address whether NK cells in the complete absence of CD45 are fully functional. In agreement with the results from the CD45ex6−/− mouse, which express CD45 at low levels, NK cells isolated from CD45ex9−/− spleenocytes have normal cytotoxic activities, as assessed by NK lysis of YAC-1 targets (Fig. 5C). However, when anti-NK1.1-induced IFN-γ production by LAKs was assessed, there was a dramatic reduction in the amount of cytokine produced by CD45ex9−/− LAKs (Fig. 5D). This decreased cytokine production was also noted for TNF-α (data not shown).

**Discussion**

The transmembrane protein tyrosine phosphatase CD45 has been shown to be critically important for the development of two populations of lymphocytes, the NK cell and thymically derived T lymphocytes (30, 31, 37, 43). In CD45ex6−/− spleens there is a marked increase in the number of NK cells with normal cytotoxic activity. As NK cell development is influenced by T cell cytokines such as IL-2, the presence of a small population of mature T cells in CD45ex6−/− mice may have an influence on the maintenance of the NK cell population in these mutant mice. Therefore, the completely CD45-deficient CD45ex9−/− mice were tested for the presence of NK cells in the spleen. Strikingly, the number of NK cells was even more dramatically increased in the spleens of CD45ex9−/− mice. The total number of NK cells was increased 4-fold over that in wild-type C57BL/6 mice, indicating that the NK cell population can develop independently of CD45. There are several potential explanations for this expansion of NK cells in the absence of CD45. First, as the cellular composition of the spleen in

**Table II. Number of iIELs in intestines of CD45-deficient mice**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Total No. of Intestinal Cells (×10^6)</th>
<th>aIEL+ (%)</th>
<th>Total No. of iIELs (×10^6)</th>
<th>CD45+ aIEL+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>3.2 ± 0.4</td>
<td>12.3 ± 1.5</td>
<td>3.9 ± 0.7</td>
<td>99.5 ± 0.2</td>
</tr>
<tr>
<td>CD45ex6−/−</td>
<td>3.6 ± 0.5</td>
<td>3.9 ± 0.8</td>
<td>1.3 ± 0.2*</td>
<td>24.8 ± 7.1*</td>
</tr>
<tr>
<td>CD45ex9−/−</td>
<td>6.5 ± 1.0</td>
<td>2.6 ± 0.5</td>
<td>1.6 ± 0.4</td>
<td>0.1 ± 0.1*</td>
</tr>
</tbody>
</table>

* Total intestinal cells were isolated as described in Materials and Methods, counted and stained with biotinylated aIEL+ mAb, followed by FITC anti-CD45 and streptavidin-PE. Percentages of cells in isolated intestinal preparations were analyzed by flow cytometry, and numbers of each population were calculated (mean ± SEM). Results are means of four separate experiments (n = 6). C57BL/6 n = 5, CD45ex6−/− n = 3, CD45ex9−/−. *, p < 0.05 for CD45ex9−/− or CD45ex6−/− vs C57BL/6 mice.
The absence of CD45 is dramatically different from that of a normal spleen, it is possible that the expansion of NK cells is being driven by an external stimulus. A second possibility is that the CD45 phosphatase and the intracellular signals it regulates are directly involved in the maintenance of NK cell homeostasis. This role may be similar to the role that CD45 plays in the down-regulation of kinase activity of Src family members during integrin-mediated adhesion in macrophages (44).

In agreement with the reported functional capacity of NK cells in the CD45ex6−/− mouse, NK cells from the spleens of CD45ex9−/− mice maintain cytotoxic activity. However, in addition to cytotoxic activity, NK cells produce a variety of cytokines, including IFN-γ, TNF-α, and GM-CSF, in response to cross-linking of NK cell activation receptors, such as NK1.1 (41, 45). As it is not currently known whether cytokine production is stimulated by precisely the same pathway that activates NK cell cytotoxicity, it was important to also investigate this aspect of NK cell function. In contrast to the normal levels of cytotoxicity seen with NK cells from the CD45ex9−/− mice, there was a dramatic loss of the capacity of NK cells to produce cytokines in response to NK1.1 cross-linking. Although the NK cell activation receptor(s) involved in YAC-1 killing is still unknown, this result implicates CD45 in either the pathway necessary to induce NK cell cytokine production or in the development of this functional capacity. Most importantly, it implies that the pathways that activate NK cell cytotoxicity and NK cell cytokine production are divergent.

The NK-T cell has recently been classified as a lymphocyte subset that shares features common with both NK cells and conventional T cells (3, 7, 28). As the NK cell population is increased in both strains of CD45-deficient mice, the presence or absence of NK-T cells was investigated. In CD45ex9−/− mice, the total number of splenic NK-T cells was significantly decreased, while a significant increase in NK-T cells was seen in the iIEL population. Our finding that the subpopulation of NK-T cells that is retained in the iIEL compartment in the absence of CD45 is a CD8+ NK-T cell supports the hypothesis that this subpopulation can develop in a thymus-independent manner. Its maintenance in CD45-deficient mice is consistent with the maintenance of other extrathymically derived populations. The functional potential of these NK-T cells has been investigated by in vivo triggering of their TCR-CD3 complex with mAb. Wild-type, C57BL/6 splenic NK-T cells can produce large amounts of IL-4 in vitro after short term in vivo stimulation with anti-CD3; however, this capacity for IL-4 secretion was completely lost in the CD45-deficient NK-T cells. This lack of rapid cytokine secretion after anti-CD3 stimulation of NK-T cells...
has been previously described for the CD8⁺ NK-T cell subpopulation, which is the predominant population of NK-T cells in the iIEL compartment (7).

Recent evidence has suggested that CD45 is not required for the extrathymic development of T lymphocytes; however, these studies used the CD45ex6⁺/⁻ mouse strain in which a subset of peripheral T cells still expresses low levels of CD45 (36). Through the use of an alternative mouse strain engineered to have a complete absence of CD45 surface expression, we have now demonstrated that it is only the extrathymically derived CD8⁺ iIEL population that is maintained in the complete absence of CD45 expression. Both classical CD8α⁺ and CD4⁺ iIELs are significantly reduced in the CD45ex9⁻/⁻ mouse intestine. This is in contrast to the previously reported increase in CD4⁺ iIELs in the CD45ex6⁺/⁻ mouse strain and demonstrates that the low level expression of surface CD45 in CD45ex6⁻/⁻ mouse iIELs has a measurable effect on the overall iIEL population. Specifically, the majority of this CD45⁺ population is CD4⁺ TCRαβ⁺, a population that is drastically reduced in the CD45ex9⁻/⁻ mouse strain (36). The precise location of extrathymic iIEL maturation has not been determined, we have not been able to identify which isoform of CD45 is used in the maturation of these CD4⁺ TCRαβ⁺ iIEL. However, previous authors have reported that iIELs from wild-type C57BL/6 mice express significant amounts of two-exon, single-exon, and zero-variable exon forms of CD45 (46). The two-
exon form was a mixture of exons 4 and 5 and exons 5 and 6, while the single-exon form solely used exon 5. This exon usage is in contrast to the exon usage seen in the thymus, which has been reported to progress from the zero variable exon form in immature thymocytes (CD4<sup>+</sup> CD8<sup>-</sup>) to single- or double-exon usage, which predominantly expresses exons 5 and 6 (47). This differential usage of CD45 isoforms could explain the presence of iIELs in the CD45<sup>6x^+/−</sup> mice, as they still have the potential to express exons 4 and 5. It should be noted that the pool of TCR-expressing lymphocytes in the spleens of both strains of CD45-deficient mice was comparable in these experiments (data not shown).

Both the CD45<sup>6x^+/−</sup> and CD45<sup>6x^+</sup> mice strains have reduced numbers of iIELs. This is in agreement with the decrease in total numbers of iIELs previously reported in older CD45<sup>6x^+</sup> mice (36). Although the iIEL of CD45<sup>6x^+</sup> mice have preferentially maintained the CD8α<sup>+</sup> population, a minor population of classic (CD8β<sup>+</sup>) iIEL can be detected. However, not all the CD8β<sup>+</sup> are Thy-1<sup>+</sup>, an indication that they also may not be thymically derived (data not shown). This is in contrast to the CD45<sup>6x^+/−</sup>, which develop minimal CD8αβ<sup>+</sup> iIELs (Table III).

Recent observations in the IL-2/15Rβ chain-deficient mouse revealed the lack of TCRβ<sup>+</sup>CD8α<sup>+</sup> iIEL (10). Taken together, these data suggest CD8α<sup>+</sup> iIEL development is dependent on the expression and function of the IL-2/15Rβ chain, but not CD45.

In summary, our findings have revealed that CD45 is not required for the development or maintenance of CD8<sup>+</sup> NK-T cells, NK cells, or CD8α<sup>+</sup> iIELs. These three populations have several common features. First, in contrast to mainstream T cells, all three of these populations have the capacity to use a thymic-dependent developmental pathway (1–5). Second, the maturation pathway of these lymphocyte subsets is severely impaired in IFN regulatory factor 1-, IL-15-, and IL-15Rα- and β-chain-deficient mice. Third, all three lymphocyte subsets require signaling through the absence of CD45, i.e., that none of these subsets requires signaling through the B7 receptor β chain.

**Acknowledgments**

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